



*Acta*

# OTO-LARYNGOLOGICA

VOL 67 JANUARY-JUNE 1969 FASC 1-6

EDITOR. C. A. HAMBERGER STOCKHOLM

CONSILIUM EDITORIUM:

DANIA: H. C. ANDERSEN H. K. KRISTENSEN N. RISKER

FENNIA: O. H. MEURMAN T. PALVA U. SIIRALA

NORVEGIA: P. BERDAL T. LEEGAARD E. STEEN

SUECIA: H. DIAMANT H. ENGSTRÖM G. HERBERTS

L. HOLMCHEN HJ. KOCH

*Almqvist & Wiksells*  
BOKTRYCKERI AKTIEBOLAG  
UPPSALA 1969

# INDEX AUCTORUM

Vol. 67

- Alpert, J. See Komatsuaki, A., Harris, H. E., Alpert, J. Cohen, B. and Andrić, R.  
See Podvinec, S., Andrić, R., Šačić, V., Mitrović, A. and Anojčić, M.
- Anojčić, M. See Podvinec, S., Andrić, R., Šačić, V., Mitrović, A. and Anojčić, M.
- Aralan, M. Modifications of the osmotic pressure of perilymph and endolymph 360
- Azzena, G. B. Rhythmical cerebellar responses to the labyrinthine stimulation 619
- Babić, L. See Gustić, B., Kraljina, Z., Poljak, Z., Končić-Carnelutti, V. and Babić, L.
- Babić, L. See Kraljina, Z., Kosković, F. and Babić, L.
- Balogh, K. See Ishii, D., Takahashi, T. and Balogh, K.
- Berdal, P. See Refsum, S. B. Jr. and Berdal, P.
- Bess, F. H. See Clack, T. D. and Bess, F. H.
- Biegy, d. B. Differential intensity sensitivity and clinical masking 428
- Bretlau, P. and Jørgensen, M. Balalev. Otoliths and osteogenetic imperfections 269
- Bretlau, P. See Chevance, L. G., Jørgensen, M. Balalev. Bretlau, P. and Chevance, J.
- Brown, J. H. and Wolfe, J. W. Adaptation to prolonged constant angular acceleration 389
- Brunetti, F. Quelques aspects particuliers de la morphologie parotidienne normale et pathologique 107
- Burlan, K., Gerstl, G. G. F. und Halder, M. EEG-computer-analyse sinuöser und sinuöser akustischer Reize 333
- Cause, J. See Chevance, L. G., Jørgensen, M. Balalev. Bretlau, P. and Cause, J.
- Chevance, L. G., Jørgensen, M. Balalev. Bretlau, P. and Cause, J. Electro-microscopic studies of the otosclerotic focus 563
- Clack, T. D. and Bess, F. H. Aural harmonics: The tone-on-tone masking VS. the beat-beat method in normal and abnormal listeners 399
- Clausen, C. Das Frequenzmaximum des kalorisch angeregten Nystagmus als Kennzeichenfunktion des geprüften Vestibularorgans 639
- Coats, A. C. The diagnostic significance of spontaneous nystagmus as observed in the lectrocnystagmographic examination 33
- Cohen, B. See Komatsuaki, A., Harris, H. E., Alpert, J. and Cohen, B.
- Daly, J. P. See Kaneko, Y. and Daly, J. P.
- Dax, S. K. and Mehra, Y. N. Caloric tests in passive vascular congestion of the labyrinth 43
- Dix, M. R. and Hood, J. D. Observations upon the nervous mechanisms of vestibular habituation 310
- Eguchi, K. See Kirikae, I., Eguchi, K., Okamoto, M. and Nakamura, K.
- Ekelund, C. and Schneider, L. B. Eosinophilic papillary cystadenoma of the larynx 467
- Engel, A. and Stahl, J. Hearing loss and caloric response in Menière's disease 57
- Enfors, B., Lind, M. and Soderborg, B. Salivary-gland scanning with 99m Technetium 650
- Ernstson, S., Lundquist, P.-G., Wedenberg, E. and Wersäll, J. Morphological changes in vestibular hair cells in a strain of the waltzing guinea pig 621
- Erorden, O. See Karalay, S., Katicoglu, S. and Erorden, O.



*Almqvist & Wiksells*  
BOKTRYCKERI AKTIEBOLAG  
UPPSALA 1969

Kristina, P and Verstraete W Isoenzymes of lactic dehydrogenase of the perilymph and the endolymph	706
Komatsu, A, Harris, H E., Alpert, J and Cohen R. Horizontal nystagmus of rhesus monkeys	523
Konić-Carnelutti, V See Guskó B., Krajina, Z., Poljak, Z., Konić-Carnelutti V and Babić, I	
Kurad, H. R. and Rattenborg, C. G. Combined action of laryngeal muscles	816
Kosković, F See Krajina, Z., Kosković F and Babić I	
Krajina, Z., Kosković F and Babić I. The bacteriology of the respiratory tract in various pathological conditions	433
Krajina, Z. See Guskó B., Krajina, Z., Poljak, Z., Konić-Carnelutti, V and Babić, I	
Krpanić J Nemanić Presbycusis, presbystia ls and preabvoamia as consequences of the analogous biological process	217
Kufner, W Cation transport and cochlear function	700
Lalig, D. Virus as the cause of rhinopharyngeal carcinoma	190
Lawrence J L. See Puharich, H K. and Lawrence J I	
Leborgne, F J See Mendoza, D Rius, M., de Stefani, E. and Leborgne F Jr	
Lidén, G and Rantkumén, A. Visual reflex cement audiometry	281
Lind, M. See Enfors, B., Lind, M. and Söderberg, B.	
Lindeman, H. H. Regional differences in sensitivity of the vestibular sensory epithelium to ototoxic antibiotics	177
Lundborg, T See Floberg, L. E., Ivarsen, B. and Lundborg, T	
Lundquist, P-G. See Ernström, J., Lundquist, P-G. Wedenberg F and Weru, J	
Lundquist, P-G. See Jahnke, V. Lundquist, P-G. and Werau, J	
Luyet, M. See Montandon, A., Huguenin, S., Rohr A., Luyet, M. and Pitteloud M	
Mehra, Y V See Das, S. K. and Mehra, Y V	
Méon, J Etude comparative de la composition chimique du mucus nasal prélevé au cours des hypersecretions de rigueur réflexe et allergique	168
Mendoza, D Rius, M., de Stefani, E. and Leborgne F Jr Experimental otosclerosis	9
Mozio, P Comportement de quelques phénomènes auditifs en hypoxie	253
Neyer zum Gottesberge A. and Stupp H F Streptomycinplegel in der perilymphe des Menschen	171
Nichle A. Recurrent-Kurzschluss-Anastomose zur Rückgewinnung der verlorenen Stimmippenmotilität	137
Mitrović K. See Podrinec, S., Andrić R., Salić V. Mitrović K. and Anojčić, M.	
Montandon, A., Huguenin, S., Rohr A., Luyet, M. and Pitteloud, M The nystagmus threshold Basis of vestibulometry	293
Münzel, M. See Yumann, H H. Naumann W H., Münzel, M. und Oeff K	
Nakajima, R. and Nakamura, T. Mast tympanoplasty with special reference to the postoperative hearing	471
Nakajima, R. See Nakajima, R. and Nakamura, T	
Nakamura, K. See Kirikae, I. Eguchi, K., Okamoto, M. and Nakamura, K	
Nakamura, T. See Nakajima, R. and Nakamura, T	
Naumann, H. H. Yumann W H. Münzel, M. und Oeff K. Zur Problematik der Resorptionsmessung der Nasenschleimhaut	122
Naumann, W H. See Naumann H H. Naumann, W H. Münzel, M. und Oeff K.	
Nishimura, T. and Takasu, T. The adrenergic innervation in the esophagus and respiratory tract of the rabbit	414
Nomura, Y. See Kirikae, I. Nomura, Y. and Hiraike, F	
Oeff K. See Naumann H H., Naumann, W H. Münzel, M. und Oeff K.	

Etholm, B. Evoked responses in the inferior colliculus, medial geniculate body and auditory cortex by single and double clicks in cats	319
Floberg L. E., Iiväta, B. and Lundborg, T. Some experiences with three hundred stapedectomized patients	501
Forrez, G. See Tyberghein, J. and Forrez, G.	
Friberg S. Jr and Kinnman J. Renal adenocarcinoma with metastases to the thyroid gland	652
Friedmann I. The innervation of the developing fowl embryo otocyst in vivo and in vitro	224
Frons, M. Ear malformations in cleidocranial dysostosis	483
Gestring G. F. See Burian, K., Gestring, G. F. und Halder M.	
Giesen M. und Klinke, R. Die Richtcharakteristikk primärer Afferenzen des Otolithenorgans bei intakter efferenter Innervation	49
Groen, J. J. Diagnostic value of lateralization ability for dichotic time differences	326
Gusté B., Krajina Z., Poljak, Z., Končić-Carnelutti, V. and Babić, I. The influence of heat on the upper respiratory tracts	150
Halder M. See Burian, K., Gestring, G. F. und Halder M.	
Hall, J. G. The cochlea and the cochlear nuclei in the bat	350
Hall, J. G. The cochlea and the cochlear nuclei in the bat	490
Harbert F., Young, I. M. and Weiss, B. G. Clinical application of intensity difference limen	435
Harris, H. E. See Komatsuaki A., Harris, H. E. Alpert, J. and Cohen B.	
Harrison M. Spencer Vestibular neuronitis	379
Hast, M. H. The primate larynx	84
Hiraike F. See Kirikae I., Nomura, Y. and Hiraike F.	
Hitselberger W. E. See House W. F. and Hitselberger W. E.	
Hood J. D. See Dix M. R. and Hood, J. D.	
House W. F. and Hitselberger W. E. The middle fossa approach for removal of small acoustic tumors	413
Huguenin S. See Montandon A., Huguenin S., Rohr A., Luyet, M. and Pitteloud, M.	
von Ilberg, Ch. and Vosteen K. H. Permeability of the inner ear membranes	165
Ishii D., Takahashi T. and Balogh, K. Glycogen in the inner ear after acoustic stimulation	513
Iiväta B. See Floberg L. E., Iiväta, B. and Lundborg T.	
Jahnke V., Lundquist, P.-G. and Wersäll, J. Some morphological aspects on sound perception in birds	583
Jeschek, J. Zum Problem der retrograden Motilitätsstörungen im Vagusbereich	142
Jørgensen M. Balslev See Bretlau P. and Jørgensen M. Balslev	
Jørgensen M. Balslev See Chevance L. G. Jørgensen M. Balslev Bretlau, P. and Cause J.	
Kaneko Y. and Daly J. F. Activity of acetylcholinesterase on the endolymphatic surface of outer hair cells	60
Kaukkanen A. See Lidén G. and Kaukkanen, A.	
Karatay S. Katircioglu, S. and Erözden O. Titration of hormone metabolites in cases of nasopharyngeal angiofibroma five years after the operation	97
Katircioglu, S. See Karatay S. Katircioglu, S. and Erözden O.	
Kawabata I. and Paparella, M. Atypical cilia in normal human and guinea pig middle ear mucosa	11
Kinnman J. See Friberg S. Jr and Kinnman J.	
Kirikae I., Nomura Y. and Hiraike F. The capillary in the human cochlea	1
Kirikae I. Eguchi K., Okamoto M. and Nakamura, K. Histopathologic changes in the auditory pathway in cases of fatal head injury	341
Klinke R. See Giesen M. und Klinke, R.	

# INDEX ACTORUM

Vol. 67

Akustischer Hitz EEG-computer-analyse, sinnloser und sinnvoller	333
Allergic information in laboratory animals, Experimental transfer of	131
Angiofibroma, Titration of hormone metabolites in cases of nasopharyngeal, Five years after the operation	07
Angular acceleration, Adaptation to prolonged constant	389
Arterial obstruction Temporary	460
Audiometry Visual reinforcement	281
Audiometry in normal hearing subjects, Cortical	24
Auditory evoked, Comportement de quelques phénomènes	200
Auditory fatigue, The absence of effects of alarm on	611
Auditory pathway in cases of fatal head injury Histopathological changes in the	241
Caloric tests in passive vascular occlusion of the labyrinth	43
Cochlea and the cochlear nuclei in the bat, The	250 and 490
Cochlea, The capillary in the human	1
Cochlea and function, Respiration of the	17
Cochlear function Cation transport	200
Cochlear nuclei in the cat, The intrinsic organization of the	302
Corti of the cat, Nerve innervation patterns in the organ of	239
Dysostosis, Ear malformations in chondrocranial	483
Electronystagmography Three derivations	303
Endolymphatic surface of outer hair cells, Activity of acetylcholinesterase on the	602
Hearing rehabilitation by means of transdermal electrotherapy in human hearing loss of sensorineural origin Preliminary report	69
Inner ear Acute degenerative changes in the sensory cells of the guinea pig following local X-ray irradiation	262
Inner ear after acoustic stimulation, Glycogen in the	573
Inner ear membranes, Permeability of the	160
Innervation in the esophagus and respiratory tract of the rabbit, The ad- renergic	444
Intensity difference limen, Clinical application of	433
Labyrinthine stimulation, Rhythmical cerebellar responses to the	610
Laryngeal muscles, Combined action of	646
Larynx, Eosinophilic papillary cystadenoma of the	467
Larynx, The primate	84
Lateralization ability for dichotic time differences, Diagnostic value of	226
Masking, Differential intensity sensitivity and clinical	428
Masking VS, The tone-on-tone, the beat-beat method in normal and abnormal hearing, Aural harmonics	399
Mastoidectomy Obliteration of the cavity in	316
Meningitis, Hearing loss and caloric responses in	57
Metastases to the thyroid gland, Renal adenocarcinoma with	332
Middle ear mucosa, Atypical cilia in normal human and guinea pig	511
Morphologie parotidienne normale et pathologique, Quelques aspects parti- culiers de la	107

- Okamoto M. See Kirikae I Eguchi K Okamoto M and Nakamura K  
 Osen Kirsten Kjellberg The intrinsic organization of the cochlear nuclei in the cat 352
- Padovan I See Pansini M and Padovan, I
- Palva T and Raunio, V The total protein of human perilymph 211
- Pansini M and Padovan I Three derivations in electronstagnography 343
- Paparella, M See Kawabata I and Paparella, M
- Perlman H B. See Tsunoo M and Perlman H B
- Pitteloud, M See Montandon, A. Huguenin S Rohr A., Luyet M and Pitteloud M
- Podvinec S., Andrić R., Savić, V Mitrović K and Anojčić M Experimental transfer of allergic information in laboratory animals 131
- Poljak, Z. See Gusić, B., Kraljina Z., Poljak, Z., Končić-Carnelutti V and Babić, I
- Präzma, J Active ion transport from the scala vestibuli into the scala media 631
- Puharich H K. and Lawrence, J L. Hearing rehabilitation by means of transdermal electrotherapy in human hearing loss of sensorineural origin. Preliminary report 69
- Rattenborg, C. C. See Konrad H R and Rattenborg C. C.
- Raunio V See Palva, T and Raunio V
- Refsum, S B Jr and Berdal, P A clinical experimental investigation of the rate of cell proliferation in human malignant tumours 101
- Rius, M See Mendoza, D., Rius, M., de Stefani, E. and Leborgne F Jr  
 Rohr A. See Montandon A Huguenin S., Rohr A., Luyet M and Pitteloud M
- Savić, V See Podvinec, S., Andrić, R Savić, V., Mitrović, K. and Anojčić M
- Schnürer L B See Ekedal, C. and Schnürer L B
- Spoendlin H Innervation patterns in the organ of Corti of the cat 239
- Stahle, J See Enander A. and Stahle, J
- de Stefani E. See Mendoza, D., Rius, M de Stefani, E. and Leborgne, F Jr
- Stupp H F See Meyer zum Gottesberge A. und Stupp H F
- Söderborg, B. See Enfors, B., Lind M and Söderborg, B.
- Takahashi T See Ishii, D., Takahashi T and Balogh K.
- Takasu T See Nishimura T and Takasu T
- Tato J M et Tato J M Quelques résultats des examens otologiques et audiolologiques des indiens sud-américains 277
- Tos, M Obliteration of the cavity in mastoidectomy 510
- Tsunoo M and Perlman H B. Respiration of the cochlea and function 17
- Tsunoo M and Perlman H B. Temporary arterial obstruction 460
- Tyberghein J and Forrez, G Cortical audiometry in normal hearing subjects 24
- Ward, W D See White Nancy C. and Ward W D
- Wedenberg E. See Ernston S Lundquist, P-G Wedenberg E. and Wersäll, J
- Weiss, B C See Harbert, F., Young, I M and Weiss, B C
- Verstraete, W See Kluydens, P and Verstraete W
- Wersäll, J See Ernston, S Lundquist P G Wedenberg, E. and Wersäll, J
- Wersäll J See Jahnke V Lundquist, P-C and Wersäll, J
- White Nancy C. and Ward W D The absence of effects of atarax on auditory fatigue 611
- Winther F O Acute degenerative changes in the inner ear sensory cells of the guinea pig following local X-ray irradiation 287
- Wolfe J W See Brown J H and Wolfe J W
- Yosteen, K H See von Ilberg Ch and Yosteen K H
- Young, I M See Harbert F., Young, I M and Weiss, B C

## THE CAPILLARY IN THE HUMAN COCHLEA

I. KIRIKAE, Y. NOMURA and F. HIRAIDE

*From the Department of Otorhinolaryngology, University of Tokyo, Tokyo, Japan*

The capillary area of eighteen aged human cochleas was studied using an alkaline phosphatase staining method. The technique is described in detail. Modes of capillary branching in the spiral lamina were different from turn to turn. The vas spirale, a loop capillary vessel beneath the turn of Corti, was long in the basal turn and short in the upper turn. The spiral lamina of the upper turn was more vascularized than that of the basal turn. A morphometric measurement showed that the ratio of capillary density in the areas of 2 mm and 3 mm was 1:2. The significance of the findings is discussed in connection with the vulnerability of the basal turn of the cochlea in sensorial-neural deafness.

It has been suspected that disturbances in the inner ear blood circulation are related to some of the inner ear diseases. Experimental work has demonstrated the labyrinth to be damaged temporarily or permanently by circulatory disturbances (Kimura & Perlman, 1956 and 1958; Perlman *et al.* 1959; Griffith, 1961; Tsunoo & Perlman, 1964).

It is difficult, however, to correlate pathologic changes in circulatory failure in the human temporal bone. Hemorrhage in the inner ear has only occasionally been reported in the leukemic temporal bone. So far, sudden deafness, which has long been thought to be of vascular origin, has demonstrated a viral infection of the labyrinth (Lindsay, 1959; Schuknecht *et al.* 1962). It is extremely difficult to trace blood vessels of the labyrinth in conventional celloidin specimens. The purpose of this paper is to present the morphology of the capillaries of the human cochlear membranous labyrinth and to discuss its functional significance using a technique we have recently developed.

### MATERIAL AND METHOD

Eighteen temporal bones from 14 patients were submitted for this study. The age of the patients ranged from 43 to 90 years. The temporal bone was removed and fixed in a 10 per cent solution of formalin methanol. The cochlea was dissected without decalcification and stained according to the following procedure:

1. A tissue was incubated in the following solution for up to 3 hours at 4°C.

Motilitätsstörungen im Vagusbereich Zum Problem der retrograden	142
Mucus nasal prélevé au cours des hyperécrétions d'origine réflexe et allergique Etude comparative de la composition chimique du	153
Nasenschleimhaut, Zur Problematik der Resorptionsmessung an der	122
Nystagmus I als Kennlinienfunktion des geprüften Vestibularorganes, Das Frequenzmaximum des kalorisch ausgelösten	639
Nystagmus as observed in the electronystagmographic examination The diagnostic significance of spontaneous	23
Nystagmus of rhesus monkeys, Horizontal	533
Nystagmus threshold The Basis of vestibulometry	293
Otocyst in vivo and in vitro, The innervation of the developing fowl embryo	224
Otolithenorgans bei intakter efferenter Innervation Die Richtcharakteristik primärer Afferenzen des	49
Otologiques et audiologiques des indiens sud américains, Quelques résultats des examens	277
Otosclerosis and osteogenesis imperfecta	269
Otosclerosis, Experimental	9
Otosclerotic focus, Electron microscopic studies of the	563
Perilymph and endolymph Modifications of the osmotic pressure of	360
Perilymph and the endolymph, Isoenzymes of lactic dehydrogenase of the	206
Perilymph The total protein of human	211
Perilymphe des Menschen Streptomycinspiegel in der	171
Presbycusis, presbystasis and presbyosmia as consequences of the analogous biological process	217
Recurrent Kurzschluss-Anastomose zur Rückgewinnung der verlorenen Stimm- lippenmotilität	137
Respiratory tracts, The influence of heat on the upper	150
Respiratory tract in various pathological conditions, The bacteriology of the	453
Responses, Evoked, in the inferior colliculus, medial geniculate body and auditory cortex by single and double clicks in cats	319
Rhinopharyngeal carcinoma. Virus as the cause of	190
Scala vestibuli into the scala media Active ion transport from the	631
Scanning with 99m Technetium, Salivary gland	650
Sound perception in birds, Some morphological aspects on	583
Stapedectomized patients, Some experiences with three hundred	501
Tumors, The middle fossa approach for removal of small acoustic	413
Tumours, A clinical experimental investigation of the rate of cell proliferation in human malignant	101
Tympanoplasty Meato- with special reference to the postoperative hearing	471
Vestibular habituation Observations upon the nervous mechanisms of	310
Vestibular hair cells in a strain of the waltzing guinea pig. Morphologic changes in	321
Vestibular neuronitis	39
Vestibular sensory epithelia to ototoxic antibiotics, Regional differences in sensitivity of the	177

## THE CAPILLARY IN THE HUMAN COCHLEA

I KIKIKAE, Y NOMURA and F HIRASIDE

From the Department of Otorhinolaryngology, University of Tokyo, Tokyo, Japan

The capillary area of eighteen aged human cochleas was studied using an alkaline phosphatase staining method. The technique I described in detail. Modes of capillary branching in the spiral lamina were different from turn to turn. The so-called spiral capillary vessel beneath the turn I of Corti, was long in the basal turn and short in the upper turn. The spiral lamina of the upper turn was more vascularized than that of the basal turn. A morphometric measurement showed that the ratio of capillary density in the areas of 33 mm and 1 mm was 1.2. The significance of the finding is discussed in connection with the vulnerability of the basal turn of the cochlea in sensorineural deafness.

It has been suspected that disturbances in the inner ear blood circulation are related to some of the inner ear diseases. Experimental work has demonstrated the labyrinth to be damaged temporarily or permanently by circulatory disturbances (Himura & Perlman, 1956 and 1958; Perlman *et al* 1959; Griffith, 1961; Tsunoo & Perlman, 1964).

It is difficult, however, to correlate pathologic changes to circulatory failure in the human temporal bone. Hemorrhage in the inner ear has only occasionally been reported in the leukemic temporal bone. So far sudden deafness, which has long been thought to be of vascular origin, has demonstrated a viral infection of the labyrinth (Lindsay 1959; Schuknecht *et al* 1962). It is extremely difficult to trace blood vessels of the labyrinth in conventional celloidin specimens. The purpose of this paper is to present the morphology of the capillaries of the human cochlear membranous labyrinth and to discuss its functional significance using a technique we have recently developed.

### MATERIAL AND METHOD

Eighteen temporal bones from 14 patients were submitted for this study. The age of the patients ranged from 45 to 90 years. The temporal bone was removed and fixed in a 10 per cent solution of formalin-methanol. The cochlea was dissected without decalcification and stained according to the following procedure:

1. A tissue was incubated in the following solution for up to 3 hours at 4°C.



Motilitätsstörungen im Vagusbereich, Zum Problem der retrograden	142
Mucus nasal prélevé au cours des hypercécérations d'origine réflexe et allergique, Etude comparative de la composition chimique du	158
Nasenschleimhaut, Zur Problematik der Resorptionsmessung an der	122
Nystagmus I als Kennlinienfunktion des geprüften Vestibularorgans, Das Frequenzmaximum des kalorisch ausgelösten	639
Nystagmus as observed in the electronystagmographic examination The diagnostic significance of spontaneous	33
Nystagmus of rhesus monkeys, Horizontal	535
Nystagmus threshold, The Basis of vestibulometry	293
Otocyst in vivo and in vitro The innervation of the developing fowl embryo	224
Otolithenorgans bei intakter efferenter Innervation Die Richtcharakteristik primärer Afferenzen des	49
Otologiques et audilogiques des indiens sud-américains, Quelques résultats des examens	277
Otosclerosis and osteogenesis imperfecta	269
Otosclerosis, Experimental	9
Otosclerotic focus, Electron microscopic studies of the	563
Perilymph and endolymph Modifications of the osmotic pressure of	360
Perilymph and the endolymph Isoenzymes of lactic dehydrogenase of the	206
Perilymph, The total protein of human	211
Perilymphe des Menschen Streptomycinspiegel in der	171
Presbycusis, presbycusis and presbycusis as consequences of the analogous biological process	217
Recurrent Kurzschluss Anastomose zur Rückgewinnung der verlorenen Stimm-lippenmotilität	137
Respiratory tracts, The influence of heat on the upper	150
Respiratory tract in various pathological conditions, The bacteriology of the	403
Responses, Evoked, in the inferior colliculus, medial geniculate body and auditory cortex by single and double clicks in cats	319
Rhinopharyngeal carcinoma. Virus as the cause of	190
Scala vestibuli into the scala media, Active ion transport from the	631
Scanning with 99m Technetium Salivary-gland	650
Sound perception in birds, Some morphological aspects on	583
Stapedectomized patients, Some experiences with three hundred	501
Tumors, The middle fossa approach for removal of small acoustic	413
Tumours, A clinical experimental investigation of the rate of cell proliferation in human malignant	101
Tympanoplasty Meato- with special reference to the postoperative hearing	471
Vestibular habituation Observations upon the nervous mechanisms of	310
Vestibular hair cells in a strain of the waltzing guinea pig, Morphologic changes in	521
Vestibular neuronitis	379
Vestibular sensory epithelia to ototoxic antibiotics, Regional differences in sensitivity of the	177



FIG. 2. Capillaries of the spiral lamina, stria, and organ of Corti of the whole left ear of a seventy-four-year-old male died of laryngeal cancer.

More branching of capillaries was seen in the upper turn of the spiral lamina than the basal. Morphometry (Weibel, 1963) was performed in an attempt to compare vascular density in different turns. A 25 mm area (500 cps) of the spiral lamina had capillaries of about 1.5 times more than that of the lower basal turn (8000 cps) (Table 2).

TABLE 1 *Vas spirale—its characteristics in different turns of the human cochlea*

	Basal turn	Upper turn
1. Length of loop	Long	Short
2. Number of anastomosing vessels	Few	Many
3. Distance between vas spirale and spiral border near the lower hair cell	Long	Short
4. Anastomosis with vessels in the spiral ligament	Not present	Present

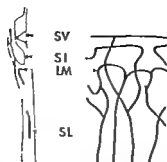


FIG. 1. Schemes of capillary distribution in the surface preparation (right) and in the ordinarily sectioned specimen (left). SV, vas spirale; SI, spiral border vessel near the inner hair cell; LM, capillary in the limbus; SL, spiral lamina.

Naphthol AS-MX phosphate	1.0 mg
Dimethylformamide	0.4 ml
Distilled water	120 ml
0.2 M propandiol buffer at pH 8.6	7.6 ml
Fast blue RR	20.0 mg

2. Washed well in running water.

3. The tissue was decalcified using a 10 per cent EDTA solution and washed in distilled water.

4. Specimens were mounted and flattened in glycerine jelly or PVP medium.

## RESULT

This paper describes only the spiral lamina vascular system, which is composed of arterioles, venules, capillaries running in the spiral lamina and the limbus, and the vas spirale. The whole specimen is shown in Fig. 2.

The vas spirale, a looping capillary vessel below the tunnel of Corti, did not run continuously all the way through the cochlea. The length of the loops was different from turn to turn. In the basal turn it was relatively longer (Fig. 3). The longest loop in this series of study measured 2 mm at a 5 mm area (8000 cps) of a cochlea from an 80-year-old patient (No. 3074). The loop was short in the upper turn (Fig. 4). In other words, there were many anastomosing vessels in the upper turn of the vas spirale and fewer in the basal turn.

Near the apical end, capillaries were sometimes found further lateral to the vas spirale. An anastomosing vessel was present, connecting the spiral lamina vascular system with the spiral ligament vascular system.

Another looping capillary was found in the vicinity of the habenula perforata. This seemed equivalent to the spiral border vessel near the inner hair cell of Smith's description (1954). The loop ran apart from the vas spirale in the basal turn and close to it in the upper turn (Figs. 3 and 4).

The characteristics of the vas spirale in different turns is summarized in Table 1.



FIG. 5. Collapse of pillars (arrows) in the spiral lamina. SY = spiral (out of focus)

TABLE 2. Comparison of capillary density in different turns of the spiral lamina

	Patient no	Side	Age	Sex	23 mm area/ 5 mm area
1	3071	r	86	f	2.0
2	3072	l	81	m	1.9
3	3069	r	87	f	1.9
4	3080	l	87	f	1.5
5	3090	r	79	m	1.2
6	3090	l	79	m	1.5
7	3091		90	f	1.5
8	3091	l	90	f	1.2
9	3092		87	m	1.0
10	3092	l	87	m	1.4
					1.5 mean

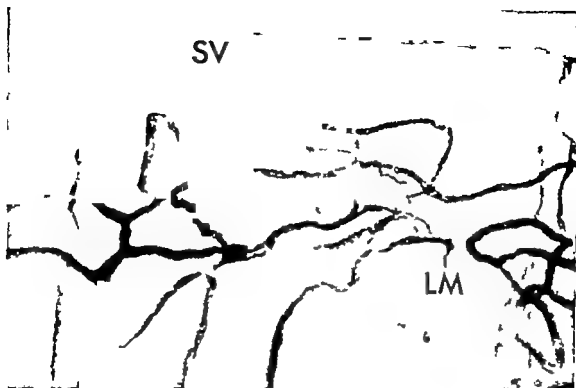


FIG. 3 Capillaries in the basal turn of the cochlea (4 mm area). Eighty-seven-year-old female. A long loop of vas spirale (560  $\mu$ ). SV Vas spirale LM limbus.

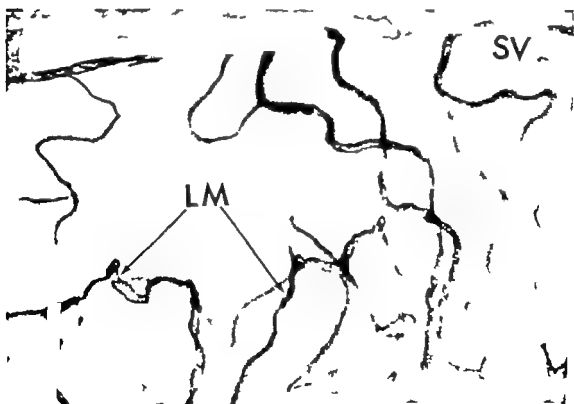


FIG. 4 Capillaries of 23 mm area. The spirale is filled for a length of 100  $\mu$ . SV Vas spirale LM, capillaries of the limbus.

discussed, the other runs near the inner hair cell. The present study shows that two spiral vessels run close in the upper turn. This, again, favors the sensory cells in the upper turn, because even if the *vas spirale* is not functioning, the spiral border vessel near the inner hair cell will compensate a part of the function of the *vas spirale* such as the diffusion of oxygen.

Morphometry definitely indicated a dense capillary network in the spiral lamina of the upper turn. Further study is being conducted to discover whether or not this is due to aging phenomena.

According to Freygang & Sokoloff (1958) blood flow rates in the cat brain parallel capillarization. Sokoloff (1961) stated that the posterior colliculus, various cortical regions, and geniculate bodies showed maximal capillary density and the highest rates of blood flow. A comparison of measurements of capillary density and succinic dehydrogenase activity in various regions of the cat brain gave a correlation coefficient of 0.88 (Friede *et al.* 1963). Taking account of these facts, scanty blood supply to the organ of Corti, where the metabolic activity is thought to be high, is contradictory if the *vas spirale* is the only source of nourishment to the organ of Corti. The oxygen tension was high near the stria vascularis. Deeper in the scala media it decreased gradually (Mishrahy *et al.* 1958). The organ of Corti is less vascularized and less oxygenated.

To sum up, when circulatory disturbances occur the sensory cells of the basal turn will be damaged more easily than those of the upper turn.

In several conditions, such as ototoxicity, presbycusis, and so on, vulnerability of the basal turn might be due to this anatomical arrangement of the capillaries of the cochlea. Although mechanisms with which the organ of Corti is involved are not understood in most circumstances, less blood circulation in the basal turn of the cochlea will definitely delay the functional restoration of damaged sensory cells.

#### ACKNOWLEDGMENT

We would like to express our thanks to Dr. Amakuni Kameyama of the Tokyo Yakuhei General Hospital for their cooperation.

#### ZUSAMMENFASSUNG

Unter Benützung von Färbung mit Alkaliphosphatase wurden die Kapillaren von III menschlichen Cochleas bei Greisen beobachtet. Die Methode wurde im einzelnen beschrieben. Die Art und Weise der kapillaren Abzweigung in der Spirallamina war verschieden. Die *vas spirale*, ein schiffenformiges Kapillargefäß unter dem Cortischen Tunnel, war lang in der Basalwindung und kurz in der Apikalwindung. Der Spirallamina der Aufwärtswindung hatte mehr Kapillaren als die der Basalwindung. Morphometrische Messungsmethode ergab, dass die Kapillaren in dem 23-mm-Spiralplattengebiet um das 1,5fache dichter waren als im 3-mm-Gebiet. Aufolge dieser Befunde wurde weiter die Leicht Verletzbarkeit der Basalwindung der Cochlea in Sensori-Neural Taubheit besprochen.

The capillary wall was generally smooth. Shrinkage and collapse of the capillary wall observed in a cochlea of very short post mortem time were seemingly evidence of a pathology of microcirculation (Fig. 5).

### DISCUSSION

The present technique demonstrates activity of alkaline phosphatase in the capillary of the cochlear membranous labyrinth. It was possible to trace the course of the capillaries, as the surrounding tissue had almost no enzyme activity. The significance of staining in the capillary wall is discussed elsewhere (Nomura & Hiraide 1968).

The functions of the capillaries in the spiral lamina vascular system are

- 1 Supply of nutrient to the organ of Corti. Lawrence (1966) has demonstrated the *vas spirale* as a source of nutrient for the organ of Corti in guinea pigs.

- 2 Maintenance of the function of the limbus. The limbus is not only anatomically related to the tectorial membrane, but has been considered to secrete the endolymph (Voldrich 1967).

- 3 Formation and absorption of the perilymph. Bast & Anson (1949) described the presence of a bony dehiscence in the lower shelf of the osseous spiral lamina.

Experiments in the human cochlea indicated that the spiral lamina was soaked with the perilymph (Nomura & Schuknecht 1965).

- 4 Nutrient supply to the nerve fibers in the spiral lamina. In addition to nourishment from the cell body, nerve fibers are possibly nourished by the surrounding medium and capillaries.

Consequently, circulatory disturbances in the vascular system will influence most of the cochlear compartments.

It is interesting to know that modes of distribution of the capillaries are different from turn to turn. When circulatory failure occurs, a long *vas spirale* in the basal turn is not beneficial to the organ of Corti. As already mentioned, the longest loop was 2 mm long in this series of study. This indicates that sensory cells serving about half of an octave are nourished by a single loop of *vas spirale* provided the vessel has a role as the source of nutrients for the organ of Corti.

Alkaline phosphatase reaction in the venous capillary in the membranous cochlea of the guinea pig was either very weak or showed no activity (Nomura & Hiraide 1968). In a loop the spiral vessel of the guinea pig had varying degrees of enzyme activity. This indicates that a segmental loop serves both as an arterial and venous capillary. Arterial blood enters into a loop, supplies oxygen, transports metabolites to and from the tissue and finally leaves the loop as venous blood from the other end.

As described by Smith, two discontinuous spiral borders are recognized in the organ of Corti and its vicinity. One is the *vas spirale* we have just

## EXPERIMENTAL OTOSCLEROSIS

*Its Causation by Ionizing Radiations*

D. MENDOZA, M. RIVERA, E. DE STEFANI and F. LEBORGNE JR

*From the Departments of Pathology and Otolaryngology Hospital de Clínicas  
Dr Mario Quintela Facultad de Medicina, Montevideo Uruguay*

Foci of osteogenesis of otosclerotic appearance were obtained in the enchondral layer of the otic capsule of dogs, using ionizing radiations. In the first dog 3 sessions were carried out over 17 days. The total dose given was 8330 rads and 7920 rads in the right and left ear respectively. The second dog underwent 6 sessions over 22 days and received a total dose of 7920 rad and 8280 rads in the right and left ear respectively. The petromastoid portions of the temporals were fixed in 10 formaldehyde and decalcified in 3% aqueous solution of trichloroacetic acid. The following histologic techniques were performed: H & E, Wilder PAS and Scharlach red. In the enchondral layer of the otic capsules of the first dog 3 otosclerotic foci were found in the walls of the internal auditory duct and of the cochlea. In the second dog a diffuse osteogenic reaction was observed. It is believed that the osteogenic foci obtained fulfill the requirements set out by various authors as demonstration of experimental otosclerotic lesions. It is furthermore contended that such foci are repair areas in a calcified enchondral layer with extensive zones of necrosis.

Few publications are available on the experimental causation of otosclerosis. Wittmack (1930) reported on the results of experiments carried out personally over several years in the course of which he provoked a venous stasis in the otic capsule of the chicken obtaining histologic images which he regarded as coincident with human otosclerosis. Mayer (1930) refuted Wittmack pointing out the absence of the characteristic arrangement in foci and that the images contended as pathologic were routine findings in normal chickens. Weber (1930) reported on experiments during which he attempted the causation of otosclerosis by inducing in dogs systemic metabolic alteration similar to that present in Recklinghausen's fibrocystic osteitis or that seen—according to the author—in Osteogenesis imperfecta. However the evidence submitted was not convincing. Kralavac (1936) carried out work along similar lines, inducing experimental poisoning with Vigantol in rats with likewise negative results. On the whole, only the periotic layer was affected by the metabolic alterations, with the production at this level of

This work was supported by Grant from Fondo de Investigación Científica de la Universidad de la República (Uruguay)



## REFERENCES

- BAST T H and ANDERSON B J 1949: *The Temporal Bone and the Ear* Charles C. Thomas, Springfield, Illinois.
- FRUETTING, W H Jr and SOKOLOFF L., 1958: Cited by R L. Friede (1966) *Topographic Brain Chemistry* Academic Press, New York.
- FRIDDE, R. L., FLEMING, L. M and KNOLLER, M 1963 A comparative mapping of enzymes involved in hexose monophosphate shunt and citric acid cycle in the brain *J Neurochem* 10 263
- GRIFFITH, A 1961 Venous obstruction of the labyrinth, some experimental and anatomical studies. *Proc Roy Soc Med* 54 917
- KIMURA R and PERLMAN H B., 1956 Extensive venous obstruction of the labyrinth. *Ann Otol* 65 832.
- 1956 Extensive venous obstruction of the labyrinth B Vestibular changes. *Ann. Otol* 65, 620
- 1958 Arterial obstruction of the labyrinth. Part 1 Cochlear changes. *Ann. Otol* 67 8
- LAWRENCE, M 1966: Effects of interference with terminal blood supply on organ of Corti *Laryngoscope* 76 1318
- LINDSEY J R., 1959 Sudden deafness due to virus infection. *Arch Otolaryng* (Chic) 69 13
- MISRAHY G A HILDBRETH, K M SHUKRABANDER, E W CLARK, L. C., and RICE, E. A 1958 Endolymphatic oxygen tension in the cochlea of the guinea pig *J.A.S.A.* 30 247
- NOMURA, Y and HIRAIWA, F 1968: Cochlear blood vessel A histochemical study *Arch Otolaryng* (Chic) 88 231
- NOMURA, Y and SCHUKNECHT H F 1965 The efferent fibers in the cochlea *Ann Otol* 74 289
- PERLMAN H B KIMURA, R and FERNANDEZ, C., 1959: Experiment on temporary obstruction of the internal auditory artery *Laryngoscope* 69 891
- SCHUKNECHT H F BENNETT, J BERKSTADT, J IGARASHI, M SINGLETON, G and RUDOL, L., 1962 The pathology of sudden deafness. *Laryngoscope* 72 1142.
- SMITH C. A 1954 Capillary areas of the membranous labyrinth. *Ann Otol* 63 433
- SOKOLOFF L., 1961 Cited by R L. Friede (1966) *Topographic Brain Chemistry* Academic Press, New York.
- TSUNOO, M and PERLMAN H 1964 Venous obstruction of the cochlea blood flow and function *Acta Otolaryng* (Stockh.) 58 127
- VOLDREICH, L., 1967 Morphology and function of the pithrium of the limbus spiralis cochleae *Acta Otolaryng* (Stockh) 63 803.
- WEIBEL, E. R 1963 *Morphometry of the Human Lung* Springer Verlag

*Dept of Otolaryngology  
University of Tokyo  
Tokyo Japan*

Received April 16 1968

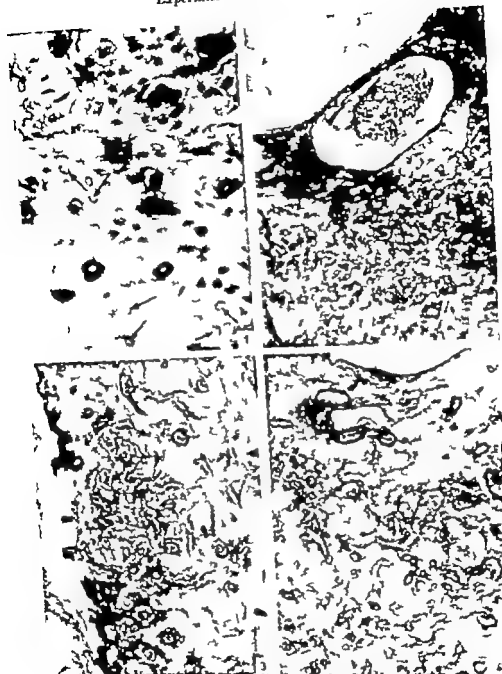


FIG. 1 First dog, left ear. Enchondral layer showing bone necrosis revealed by the presence of (least) rows of empty lacunae, some of them indicated by arrows. H & E. 100

FIG. 2 First dog, left ear. Otosclerosis-like otosclerotic focus. Note that the focus situated in dermalized one of the enchondral layer. H & E. 20

FIGS. 3, 4 and 5. First dog, right ear. Dermalized otosclerotic foci showing marked histological similarities with human otosclerotic foci. H & E. 20

lesions similar to fibrous dysplasia the enchondral layer remaining uninvolved. Lastly Ballucci & Wolff (1960) using electro-coagulation provoked vascular lesions in the otic capsule of animals "with some of the features of human otosclerosis. Upon reviewing material from experiments in rats, intended to cause osteomyelitis in their diaphyses we noticed areas of bone necrosis invaded by foci of osteogenesis similar to those seen in man. This observation prompted us to carry out experiments at the level of the otic capsule itself using various injuring agents. At an early phase chemical agents (sodium morrhuate) and physical ones (ultrasound) were employed in rabbits. Results were variable, attention being drawn to the fact that with the ultrasound there appeared areas of devitalized bone tissue with fatty degeneration of the osteocyte and a moderate osteogenesis at the level of the marrow spaces. Kristensen & Balslev-Jørgensen (1961) found a relationship between otosclerosis and irradiation and suggested that otosclerosis might be brought about experimentally by using radiation as an injuring agent. We have taken up their suggestion the following being a report of the results obtained in dogs.

#### MATERIAL AND METHODS

The inner ear of two adult dogs was irradiated with a Cobalt 60 beam therapy unit using a single right lateral  $4 \times 4$  cm field. The animals were treated twice weekly under general anesthesia with sodium pentobarbital intraperitoneally. The first dog 3 year old and weighing 4 kg received 1670 rads to the right ear and 1100 rads to the left per treatment. This dog died on the 18th day as a result of cerebral radiation injury (necropsy finding) after having received a total dose of 8330 rads to the right ear and 5000 rads to the left in  $2\frac{1}{2}$  weeks. The second animal 4 years old and weighing 11 kg was treated in the same way but received a lower dose per treatment 1320 rads and 880 rads to the right and left ear respectively. The total dose was 7920 rads and 5280 rads in  $3\frac{1}{2}$  weeks. The animal was sacrificed 48 hours after the last treatment. The temporal bones of both dogs were decalcified in 5% trichloroacetic acid following fixation in 10% formaldehyde. The petrous bones were sectioned perpendicularly to the large axis and the fragments of otic capsule thus obtained were embedded in paraffin blocks, other fragments being set aside for frozen sections. The following staining techniques were used H.E., I.A.S., Wilder and Searlach red. Six labyrinthine capsules of normal adult dogs were used as controls.

#### RESULTS

In the otic capsules of the normal adult dogs it was possible to observe a vitalized enchondral layer patent blood vessels and a few empty lacunae the number of cartilaginous remnants being smaller than in the human enchondral layer. Fatty degeneration of the osteocyte was of rare occur



rence. On other hand the irradiated animals exhibited marked pathological alterations. In the otic capsules of the first irradiated dog there were observed areas of bone necrosis involving the enchondral and periosteal layers, recognizable by the presence of extensive areas of empty lacunae. The techniques for demonstration of fats revealed the existence of sudanophilia in the osteocyte and in the empty lacunae a sign of fatty degeneration at that site. Havers canals were seen smaller in diameter resulting from apposition of an intensely basophilic substance leading to eventual vascular obliteration due to extrinsic compression. On the whole, these pathologic alterations resulted in a histologic pattern similar to that observed in the normal human otic capsule (Fig. 1). As a salient feature stress is laid on the appearance of osteogenetic foci of an "otosclerotic" aspect (Fig. 2). The osteogenic areas derived from the invasion of the enchondral layer by blood vessels stemming from the periosteal layer. The three foci observed were entirely confined to the enchondral layer. One of them (left ear) lay within the wall of the internal auditory duct and the remaining two (right ear) within the cochlear wall. Following decalcification one of the right ear focus could be seen with the naked eye on the cut surface as a minute whitish area against the greyish background of the enchondral layer. Microscopically the newly formed bone tissue was made up of bone trabeculae arranged in a disorderly erratic, non laminillar fashion separated by spaces occupied by connective vascular tissue. The size of these spaces was variable depending on the existing osteoblastic activity. In the oldest focus the bone tissue was of a sclerous appearance its vitality being nevertheless preserved. In the more recent foci it was possible to observe a predominance of bone resorption together with an intense vascularization of the area concerned (Figs. 3, 4, 5 and 6). These osteogenic areas were markedly PAS-positive the more so in the oldest focus. With the Wilder stain an erratic, non reshaped arrangement of the collagenous fibrils was seen the pattern assumed being that of bone tissue of inter-crossed fibers. These areas were in contrast with the adjacent bone tissue characterized by a laminillar arrangement of its fibers.

In sections deriving from the second dog there was a diffuse osteogenic activity involving all the layers consisting of overlapping of young bone

FIG. 6 Detail of photomicrograph 5, showing the newly formed bone tissue of sclerotic-like coloring and substituted by the decalcified enchondral layer. The focus is clearly delimited. H & E. 80

FIG. 7 Second dog, left ear. Not the marked osteogenic activity round the blood vessel with fibrillar images. Extent of empty lacunae may be observed. H & E. 80

FIG. 8 Second dog, left ear. Osteogenic area developed in decalcified enchondral layer. Note the multiplicity of wide lumina and channels in digested enchondral layer from the periosteal. H & E. 80

the effect of heavy irradiation of adult bone tissue is the production of an aseptic bone necrosis. The necrosed zones—recognizable by the presence of empty lacunae—appear arranged in irregular areas rarely is the demarcation between necrosed and live tissue definitely clear. The sequestration of necrosed bone does not take place, except for the occurrence of secondary infection. There is no general agreement on the causative mechanism of the necrosis.

Our own belief in agreement with Trueta (1963) is that the disintegration products of the necrotic cells excite a reparative osteogenesis originating in the perivascular vessels. Hence we contend that the osteogenic foci may be interpreted as repair areas at the level of a partially necrosed enchondral layer. For the development of the osteogenic foci, the occurrence of severe necrosis in the otic capsule is an essential pre-requisite. This contention is based on the fact that in the second animal there appeared no clear-cut foci, but rather a diffuse osteogenic reaction. A finding, we believe due to the absence of severe necrosis. Very likely failure in obtaining necrosis may be imputed to the administration of a somewhat smaller more fractionated dose.<sup>1</sup>

### ZUSAMMENFASSUNG

Die Verfasser erzeugten unter Verwendung ionisierender Strahlungen osteogenische Zonen otosklerotischen Aussehens in der enchondralen Schicht der Labyrinthkapseln bei Hunden. Das erste Versuchstier wurde 5mal in 18 Tagen bestrahlt, wobei die gesamte Dosis 8330 r im rechten und 7920 r im linken Ohr betrug. Das zweite Versuchstier wurde 6mal in 22 Tagen bestrahlt. In diesem Fall betrug die gesamte Dosis 7920 r im rechten und 5280 r im linken Ohr. Die temporalen Knochen wurden in 10% Formaldehyd fixiert und mittels einer 5%igen Trichloressigsäure entkalkt. Folgende histologische Techniken wurden angewandt: H. E., Wilder PAS und Scharlach rot. In der enchondralen Schicht der Labyrinthkapseln des ersten Hundes wurden 3 otosklerotische Zonen an der Wand des Innenohrkanals und der Schnecke gefunden. Beim zweiten Hund wurde eine diffuse osteogenische Reaktion beobachtet. Die Verfasser glauben, dass die osteogenischen Zonen die Vorgänger entsprechen, die verschiedene Autoren als massgebend für experimentell Otosklerose betrachten und sind außerdem der Meinung, dass die osteogenischen Herde Erneuerungsgewebe darstellen, die sich in ihrer lebensunfähigen enchondralen Schicht mit ausgedehnten Nekroseherden entwickeln.

### REFERENCES

- ALTMAN, F. 1960 *Histopathology and Etiology of Otosclerosis: A Critical Review* (Otosclerosis International Symposium, Ford Hospital, Detroit) p. 13, Little Brown & Co.

After reading the original, third dog was irradiated with total dose of 18,000 r in the right ear and 8000 r in the left. We found osteogenic foci of otosclerotic pattern. This occurs altogether similar to those described in the paper and was situated in the enchondral layer of the right labyrinthine capsule.

tissue or of osteoid tissue in areas formerly reabsorbed by osteolysis. These areas of newly formed bone were separated from one another by old bone tissue. While no osteogenetic foci similar to those seen in the first dog were found, formation of new bone was evident and the presence of finger like images consisting of a central vessel from which satellite vessels surrounded by "blue mantles" radiated was of frequent occurrence (Figs 7 and 8).

It should be pointed out that these images have been regarded by Nager (1939) as representing the initial stage of otosclerosis.

## DISCUSSION

To date none of the attempts to produce experimental otosclerosis have attained any reasonable degree of success. Available work in the field has failed to meet the requirements which identify the otosclerosis lesions as stated by Altmann (1960), Nager (1939) and Mayer (1932).

(a) Lesions should be focal, non-diffuse and confined to the labyrinthine capsule.

(b) The assumed causative factor should be capable of producing foci not only in the area of the oval window but in all the typical localization, including the internal auditory duct and the semicircular ducts.

(c) Due regard should be given to the difference, so far as the histologic picture is concerned, between the human and the animal otic capsule, only that of monkeys and to some extent, of dogs bear resemblance with the human otic capsule.

We believe that our results closely meet such requirements. Firstly, we chose the dog as the experimental animal; under normal conditions the otic capsule of the dog is rather similar to that of man and after heavy irradiation it assumes a devitalized appearance (areas of empty lacunae, fatty degeneration of the osteocyte). In accordance with Nager (1939) and our own studies (1961 and 1966) this devitalized aspect is precisely one of the outstanding features of the enchondral layer of the human otic capsule. Secondly, we obtained focal lesions, neatly distinct from the adjoining bone tissue. Thirdly, the foci, which apparently derived originally from the vessels stemming from the periosteal layer, developed exclusively in the enchondral layer, one of them in the wall of the internal auditory duct and the others in the cochlea. It is worth stressing that no cases of spontaneous otosclerosis in animals have so far been reported according to Weber (1930) and Kristensen & Balslev-Jørgensen (1967). Furthermore, in accordance with our pathogenic conception of otosclerosis, mammals cannot have it as their otic capsule is always well vitalized. As a result, one should discard the possibility of spontaneous otosclerosis in an irradiated dog or that irradiation has activated latent otosclerotic foci (Kristensen & Balslev-Jørgensen 1967). It is common knowledge (Dunlap 1966; Moss, 1950) that

## RESPIRATION OF THE COCHLEA AND FUNCTION

M. TECNOO and H. E. PERLMAN

From the Division of Otolaryngology Department of Surgery the University of Chicago Chicago Ill U.S.A

Brief reversible depression of cochlear respiration was produced in the guinea pig with 0.013 cc of 0.2 mM sodium cyanide perfused into the scala tympani of the basal turn for 80 seconds. Brief reversible depression of cochlear respiration was also produced by systemic hypoxia (respiration with 5% oxygen). When cyanide perfusion was combined with hypoxia, cochlear respiration and function were more severely affected. The effects of cyanide and hypoxia on the terminal cytochrome oxidase of the respiratory chain of enzymes in mitochondria are reviewed.

Investigations of the function of cochlear cells are guided by knowledge of the reaction of oxygen and metabolic inhibitors with respiratory enzymes. The rate of cell respiration for normal function reflected in oxygen utilization and reduction of pyridine nucleotide varies widely. For cochlear cells it can be defined as the lowest level of oxygen concentration of the blood and the slowest blood flow rate that is compatible with normal function.

The activity of the respiratory enzymes (i.e. the terminal cytochrome oxidase) can be controlled by graded hypoxia or by enzyme poisons. In the experiments described below respiration and function of the cochlear cells were depressed in this manner. To avoid systemic effects, the enzyme poisons (cyanide and azide) were perfused into the cochlea.

### METHOD

In addition to previously described procedures, perfusion of the perilymphatic space in the guinea pig was accomplished by a 200  $\mu$  diameter polyethylene cannula tightly fixed in the scala tympani of the basal turn. Another hole near the helicotrema allowed the perfusion fluid to escape. Perfusion was maintained for 80 seconds at controlled rates with a Harvard perfusion pump. Perfusion fluids included 0.02, 0.2, and 2.0 mM sodium cyanide in Ringer's solution, and 0.13 and 1.5 mM sodium azide in Ringer's solution. Systemic anoxia was produced by respiration with 5% oxygen. The effect of pH changes on cochlear function was examined with Ringer's solution.

This work was supported in part by Public Health Service Grant XE00263-1A, Clinical Research Center Grant YB03338-07 from NIH, and the Douglas Smith Foundation for Medical Research of the University of Chicago.



- BELLUCCI, R. J. and WOLFF H. 1960 Experimental trauma. Some effects to the otic capsule and their possible significance *Arch Otolaryng* (Chic.) 71: 224.
- DIMLER, CH. E., 1966: *Effect of Radiation Pathology* (ed. Anderson) p. 188. C. V. Mosby St. Louis.
- KRAINE, W. 1936 Veränderungen des Schläfenbeins der Ratte bei Vig. nt Ivergiftung. *Machr. Ohrenheilk.* 70: 6.
- KRISTENSEN, H. K., and BALSLEV-JØRGENSEN, M. 1967: Irradiation and otosclerosis. *Acta Otolaryng* (Stockh.) 88: 114.
- MAYER, O. 1932: Referat über pathologische Anat. u. Pathogenese der Otosklerose II. *Cong. Intern. O. R. L. Madrid* 1: 7.
- MENDOZA, D. and RITA, M. 1966 Histology of the chondral layer of the human otic capsule. Area of devitalized and necrotic bone. *Acta Otolaryng* (Stockh.) 87: 93.
- MOSE, W. T. 1959: *Theapeutic Radiology Ratte n le Technique Results* p. 346. C. V. Mosby St. Louis.
- NAGER, F. R. 1939: Pathology of the labyrinthine capsule and its clinical significance. In *Medicine of the Ear* (ed. Fowler) p. 237. Nelson, New York.
- RITA, M. and MENDOZA, D. 1961 Nueva teoría sobre la etiopatología de la otosclerosis: área de osteogénesis en la cápsula laberíntica parcialmente necrosada. *An. Otorinolaring* (Uruguay) 31: 63.
- TRUETA, J. 1963: The role of the capsule in osteogenesis. *J. Bone Joint Surg* (Amer.) 45B: 402.
- WEBER, M. 1930: Description of otosclerosis. Theory of its experimental reproduction. *Arch Otolaryng* (Chic.) 12: 608.
- WITTMACK, H., 1930: Über otogenes experimentelles Hühnerotosklerose. *Acta Otolaryng* (Stockh.) 14: 226.

D. Mendoza, M. D. Dept. of Pathology  
Hospital d. Clinicas Dr. Manuel Quintela  
Facultad de Medicina Montevideo  
Uruguay

Received March 1968

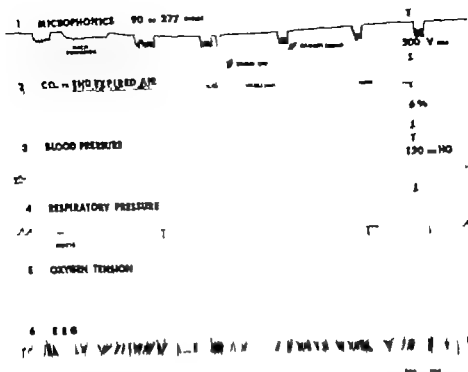


FIG. 1. The reversible effect of infusion of 0.013 cc of sodium cyanide (0.2 mM) into the scala tympani of the basal turn delivered for 80 seconds on cochlear microphonic output for a tone of 277 cycles per second at 90 dB. The systematic effects are produced and after blood flow is not changed. After a drop in microphonic output during infusion is followed by a slight temporary increase in perilymph pressure. After 2. Airborne sound from the camera and light source in the open balla apparatus (briefly and reversibly) the conduction system stimulated by the sound delivered through the ligament of the external canal.

the cyanide and azide solutions used exceeded this alkalinity. Thus, the changes in microphonic output produced with these poisons could be assessed.

The next problem was to explore the concentration of cyanide and azide needed to alter the microphonic response in a reversible manner. The duration of injections was also important in this respect. With 80 seconds of perfusion at 0.01 cc/min, a quantity was delivered so that the normal perilymph of the scala tympani was replaced by the perfusion fluid. For brief reversible effects on the microphonic output, a concentration of 0.2 mM sodium cyanide was found effective. Solutions of Na cyanide at 0.02 mM and sodium azide at 0.15 and 1.5 mM did not effect microphonic output. The reversible effect of cyanide on microphonic outputs is indicated in Table I and in Fig. 1. An initial RMS value of 390  $\mu$  volts drops to 26  $\mu$  V one minute after infusion at p.s. Recovery is complete in seven

TABLE 1 *Effect of NaCN and 5% oxygen on microphonics (RMS) blood flow and oxygen tension*

Changes in blood flow and oxygen tension expressed in per cent of base line

Time	Base line	Minutes after				
		1	2.5	4	5.5	7
<i>0 mM NaCN perfusion of 0.613 cc</i>						
Microphonics	390	285	245	325	355	390
Blood flow	100	120	110	108	98	104
Oxygen tension	100	100	100	100	100	100

Time	Base line	During	Minutes after		
			1	3	5
<i>5% O<sub>2</sub> inhalation</i>					
Microphonics	340	290	340	340	340
Blood flow	100	137	144	121	108
Oxygen tension	100	26	36	0	90
<i>Both procedures</i>					
Microphonics	325	160	160	300	325
Blood flow	100	121	144	124	110
Oxygen tension	100	63	72	90	95

of various pH up to 8.8 perfused into the perilymph. This corresponded to the alkalinity of the strongest (2.0 mM) sodium cyanide solution used for cochlear perfusion.

## RESULT

A number of problems are involved in the delivery of these respiratory enzyme poisons directly into the perilymph. The site of injection is important. Injection into the scala vestibuli may damage Reissner's membrane with sudden permanent loss of microphonic response. The rate of injection is important since too rapid flow results in increased pressure in the cochlea. This affects basilar membrane as well as footplate vibration and thus temporarily reduces microphonic output. With fluid moving at a rate of 0.01 cc/min into the scala tympani of the basal turn and out through the apical turn a temporary slight depression in microphonic was produced with no change in stria blood flow. Injections at this rate could be repeated many times without damage. The fluid used for solution of cyanide and azide is important. No changes in microphonic response were noted when mammalian Ringer's solution was perfused in this manner. The alkaline nature of cyanide and azide solutions required that the effect of pH changes of the Ringer's solution be examined. No alteration in microphonic response was seen when Ringer's solution up to a pH of 8.8 was perfused. None of

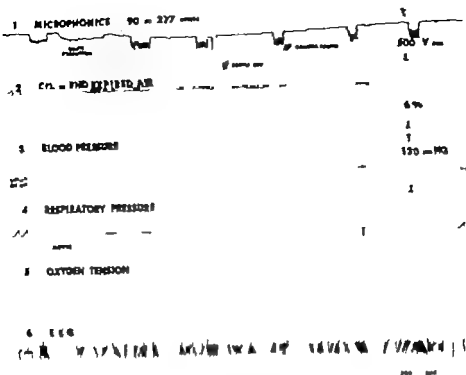


FIG. 1. The reversible effect of infusion of 0.013 cc of sodium cyanide (0.2 mV) into the scala tympani of the basal turn delivered in 80 seconds on cochlear microphonic output for sustained 277 cycle tone at 90 dB. No systematic effects are produced and arterial blood flow is not changed. Note 1. A drop in microphonic output during infusion is due to a slight temporary increase in perilymph pressure. Note 2. Airborne sound from the camera and light source to the open bulla compresses (briefly and reversibly) the eardrum (no system stimulated by the sound delivered through tubing into the external canal).

the cyanide and azide solutions used exceeded this alkalinity. Thus, the changes in microphonic output produced with these poisons could be assessed.

The next problem was to explore the concentration of cyanide and azide needed to alter the microphonic response in a reversible manner. The duration of infusions was also important in this respect. With 80 seconds of perfusion at 0.01 cc/min, a quantity was delivered so that the normal perilymph of the scala tympani was replaced by the perfusion fluid. For brief reversible effects on the microphonic output, a concentration of 0.2 mV sodium cyanide was found effective. Solutions of Na cyanide at 0.02 mV and sodium azide at 0.15 and 1.5 mV did not effect microphonic output. The reversible effect of cyanide on microphonic outputs is indicated in Table 1 and in Fig. 1. An initial RMS value of 300  $\mu$  volts drops to 265  $\mu$  V one minute after infusion stops. Recovery is complete in seven

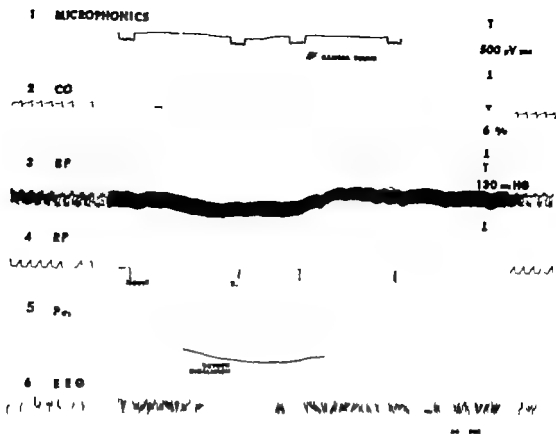


FIG. 2. The effect of 5%  $O_2$  hypoxia (5% oxygen breathing).

minutes. Simultaneous measurement of blood flow velocity in the stria and of perilymph oxygen tension revealed no significant change. Perfusion with higher concentration of cyanide (2.0 mM) produced a marked more sustained drop in microphonic output. Unlike the 0.2 mM solutions, these higher concentrations of cyanide directly effect the performance of the polarographic electrodes by producing small faradic currents so that simultaneous values of perilymph oxygen tension could not be recorded by this method.

A significant reduction in oxygen delivery to the cochlea, to produce small reversible changes in perilymph oxygen tension and in microphonic output, was found possible by respiring the animal with 5% oxygen.

Other effects of hypoxia are also recorded such as flattening of the EEG, increased cochlear blood flow drop in carotid blood pressure and in  $CO_2$  of end expired air.

Thus inhalation of 5% oxygen for 120 seconds results in a reversible drop in microphonic output from an initial RMS value of 340  $\mu V$  to 290  $\mu V$ . Normal values are regained one minute after return to air breathing (20% oxygen) (Fig. 2). Respiration with 5% oxygen for five minutes does not further increase the effect. At the same time strial blood flow is increased 37%. Flow returns to normal five minutes after resumption of air breathing. Perilymph oxygen tension drops to 26% of normal when the animal is respired with 5% oxygen and returns to normal level five minutes after resuming air breathing.

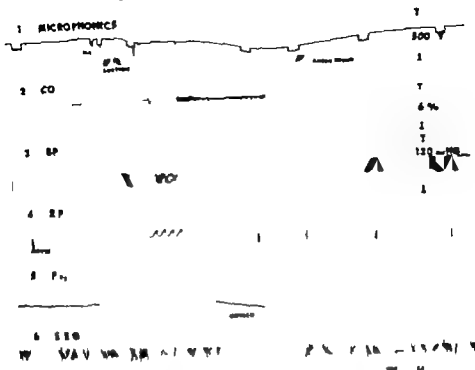


FIG. 2. The more severe but reversible effect of combining local cyanide infusion with systemic hypoxia on cochlear function (microphonic output).

The additive effect of cochlear hypoxia and cyanide was noted when inhalation for two minutes of 5%  $O_2$  immediately followed perfusion of the scala tympani with cyanide. Microphonic output dropped from an initial value of  $325 \mu V$  to  $160 \mu V$ . Normal values were regained five minutes after  $O_2$  breathing was resumed.

Changes in cochlear blood flow were similar to those produced by hypoxia alone. However polarographic values for perilymph oxygen tension were less depressed than with hypoxia. Changes in other parameters monitored were similar to those produced by 5%  $O_2$  inhalation (Fig. 3).

### DISCUSSION

Cyanide has been used widely as a metabolic inhibitor to alter function and oxygen uptake of excised nerves.

Details of the effect of cyanide on the cochlea are limited. Davis (1955) reports that small doses of cyanide in any one of the three scala promptly abolished the cochlear microphonics, endolymphatic potential, and action potentials. Rapid (25 minutes) irreversible deterioration of the microphonic

Kodishi & Kelsey (1968) found that ten microliters of 1% CN (50 mg. males) perfused into the scala tympani in 3 to 5 minutes promptly caused a severe drop in cochlear microphonics, action potentials, endolymphatic potentials and probably depolarization of the cells in the organ of Corti.

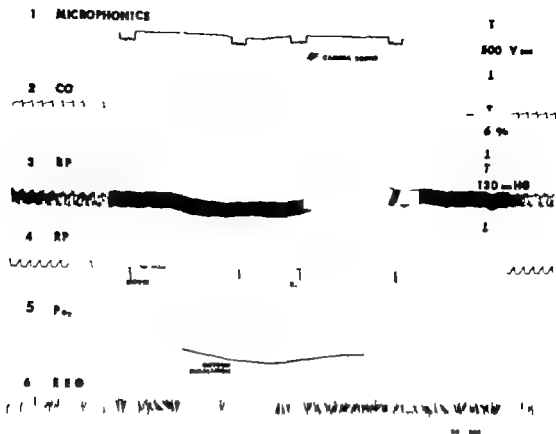


FIG. 2. The effect of systemic hypoxia (5% oxygen breathing).

minutes. Simultaneous measurement of blood flow velocity in the stria and of perilymph oxygen tension revealed no significant change. Perfusion with higher concentration of cyanide (2.0 mM) produced a marked more sustained drop in microphonic output. Unlike the 0.2 mM solutions, these higher concentrations of cyanide directly effect the performance of the polarographic electrodes by producing small faradic currents so that simultaneous values of perilymph oxygen tension could not be recorded by this method.

A significant reduction in oxygen delivery to the cochlea to produce small reversible changes in perilymph oxygen tension and in microphonic output, was found possible by respiring the animal with 5% oxygen.

Other effects of hypoxia are also recorded, such as flattening of the EEG, increased cochlear blood flow, drop in carotid blood pressure and in  $\text{CO}_2$  of end expired air.

Thus inhalation of 5% oxygen for 120 seconds results in a reversible drop in microphonic output from an initial RMS value of 340  $\mu\text{V}$  to 200  $\mu\text{V}$ . Normal values are regained one minute after return to air breathing (20% oxygen) (Fig. 2). Respiration with 5% oxygen for five minutes does not further increase the effect. At the same time stria blood flow is increased 37%. Flow returns to normal five minutes after resumption of air breathing. Perilymph oxygen tension drops to 20% of normal when the animal is respired with 5% oxygen and returns to normal level five minutes after resuming air breathing.

is inactivated by cyanide. In the cochlea this is reflected in a greater reduction (50%) in function (microphonic output). This was reversible and the time needed to restore normal function was about the same as with 5% oxygen (7 minutes). On the other hand, oxygen tension in the perilymph did not drop to the same degree as with hypoxia alone. This may be due to a further decrease in oxygen utilization by the respiratory enzymes in cochlear cells. The additive effect of hypoxia and cyanide on the brain is reported by Levine & Stypulkowski (1959) who administered cyanide and ligated one carotid in the rat. They found more histological evidence of cerebral degeneration on the side of the carotid ligation.

## ZUSAMMENFASSUNG

Die reversible Abnahme der Mikrophonenpotentiale wird durch endocochleäre (Scala tympani) Durchströmung von 0,2 mM NaCN gefunden. 5-%ige O<sub>2</sub>-Inhalation kurz nach Durchströmung von NaCN verstärkt diese Abnahme der Mikrophonenpotentiale. Die Effekte von NaCN und Hypoxia auf Zytochromoxidase werden diskutiert.

## REFERENCES

- BLOOM, B. M., FRANKS, J., HILLMAN, H., KENNEDY, H., and MCCREARY, J. 1961: A study of cerebrospinal fluid oxygen tension. *Arch. Neurol. (Chic.)* 4: 311.
- BOEVEN, H., and TRAMER, R. 1963: Die Beeinflussung der Mikrophonenpotentiale durch endocochleäre Applikation von Zodaenol. *Experientia* 19: 412.
- CHEN, B., COHEN, P. J., JOHNS, P. and SCHWARTZ, B. 1962: Intracellular oxidation-reduction states in vivo. *Science* 127: 499.
- COHEN, P. J. and ALKANA, S. C. 1967: Effects of hypoxia and normobarbital on cerebral blood flow and metabolism in conscious man. *J. Appl. Physiol.* 23: 163.
- DILLI, H. 1957: Biophysics and physiology of the inner ear. *Physiol. Rev.* 37: 32.
- KOHN, T. and KILPATRICK, E. 1968: Effect of Cyanide on Cochlear Potentials. *Acta Otolaryng. (Stockh.)* 63: 381.
- LEVINE, S. and STYPUKOWSKI, W. 1959: Effect of ischemia on cyanide encephalopathy. *Neurology* 9: 407.
- NECK, E. A. and SARABANANDAN, E. W. 1961: Studies on the endolymphatic potentials of the guinea pig cochlea. *J. Acoust. Soc. Am.* 33: 922.
- ROSEN, M., FERNANDEZ, F. A., and KILPATRICK, E. 1963: Increase of cerebral blood flow produced by low dosage of cyanide dose. *J. Physiol.* 204: 309.
- WALLER, A. A. and WICKSTEAD, M. D. 1947: Study in cyanide. Sequence of changes of activity induced in various parts of the central nervous system. *J. Neuropath. Exp. Neurol.* 6: 281.
- WEISS, E. G., LEVINE, S., KENNEDY, H. W. and STRATTON, C. B. 1959: The effect of oxygen deprivation upon the cochlear potentials. *Ann. N.Y. Acad. Sci.* 119: 179.
- WALLER, A. J. and KILPATRICK, E. 1966: Autoregulation of intracellular  $\text{pO}_2$  in skeletal muscle. *Fed. Proc.* 25: 2.

H. B. P. Ivers, M.D.  
950 East 58th Street  
Chicago, Ill. 60621

Received May 12, 1968



output in the cat was produced by Bornschein & Thalmann (1963) with 1 to 2 cc of 0.2% iodoacetate perfused into the perilymphatic space. A temporary reversible drop in output was also noted during infusion of fluids. This was attributed to increased intracochlear pressure.

The effects of cyanide perfusion on cerebral function are also of interest. Ward & Wheatley (1947) found that reversible obliteration of the EEG is produced by intravenous administration of 0.5 to 1.2 mg/kg NaCN. Russek *et al* (1963) measured increases in cerebral blood flow with small doses of cyanide in cats and rabbits. They found no increase in the arteriovenous oxygen difference suggesting that respiration of the brain was increased. On the other hand Bloor *et al* (1961) concluded that the intracarotid injection of cyanide (0.75 mg) in the monkey decreases respiration (oxygen utilization) of the brain since polarographic values for oxygen tension rose in the sagittal sinus, cerebral cortex, and cerebrospinal fluid.

**B Hypoxia** Normal cell function can be maintained with intracellular oxygen tensions of a few millimeters of mercury. With a microelectrode in muscle cells, Whalen & Nair (1966) obtained polarographic  $pO_2$  values of about 3 mm Hg.

The inspired oxygen concentration that causes a decrease in oxygen utilization by the cell is designated by Chance *et al* (1962) as the critical oxygen tension. He found that respiration of cells on the rat brain cortex is slightly (10%) reduced when the animal is respired with 8% oxygen. When 4% oxygen was used cell respiration dropped by about half. Recent studies by Cohen *et al* (1967) indicate that respiration with 7% oxygen affects aerobic cerebral metabolism in conscious man. Despite an increase in cerebral blood flow the proportion of glucose undergoing complete oxidation was reduced and excess lactate appeared in the jugular venous blood. A critical oxygen tension may be considered for the cochlea. Weyer *et al* (1949) found that cochlear function (microphonic response) begins to decrease when the animal is respired with 4% oxygen mixtures. A similar value was found by Rice & Shinabarger (1961). We used brief (120 sec) periods of cochlear hypoxia (5% oxygen) and regularly produced a controlled, small reversible drop in cochlear function. Longer exposures to 5% oxygen (300 sec) did not further reduce the microphonic response. A measured increase in strial blood flow rate was due to relaxation of vascular smooth muscle and not to increase in perfusion pressure. The carotid blood pressure was reduced at this time while pulse pressure remained constant. General reduction in aerobic metabolism of the animal on 5% oxygen is indicated in the recorded drop in  $CO_2$  tension of end expired air. With return to air breathing, a brief period of supernormal  $CO_2$  production reflects a general increase in metabolic activity. While 5% oxygen mixtures produce a small drop in cochlear function it has a strong effect on cerebral function (E.E.G. frontal lobe becomes flat).

**C Hypoxia combined with cyanide perfusion** Greater effects of hypoxia on cell respiration is noted when some of the terminal cytochrome oxidase

is a mere physical problem. In this objective measuring technique, the cooperation of the subject is superfluous: the corticofugal paths are not tested. Moreover, the presence of an objective cortical reaction after acoustic stimulation does not necessarily correspond to the conscious perception of the administered stimulus. With a case of obvious hysterical deafness, which we were fortunate enough to follow accurately, we always registered normal reactions with the cortical audiogram, where the psychoacoustically determined thresholds for pure tones remained 60 dB below normal and the speech discrimination was very poor for some three weeks. The subjective "restitutio ad integrum" of audition that appeared after 1 month did not change the cortical audiogram in any way. Many investigators proved the influence of the psychic condition of the subject (attention, indifference, drowsiness, or sleep) on the obtained curves. The shape of the registered potential changes ultimately depends upon the technique. During the first 50 s after the beginning of the acoustic stimulation the myogenic potential of the sonomotor reflex (Bickford *et al.* 1964; Cody *et al.* 1964; Mast 1965) is registered. It originates in the neck muscles, the *musculus temporalis*, and the external ear muscles and is supposed to be connected with the functional condition of the vestibular apparatus. This potential can be derived in general from the skin covering the striate muscles. It increases with artificial increase of mechanical tension of the neighbouring muscles—especially of the neck muscles—and disappears almost completely with total muscle relaxation. In a recent publication Rahm *et al.* (1967) assume that this early component is also of cochlear origin.

The beginning of the proper slow evoked potential is somehow masked in the registration by the end of the myogenic potential. It concerns a series of alternately vertex negative (N) and vertex positive (P) peaks that correspond with the N potential produced by acoustic, tactile, or visual stimuli (Davis, *et al.* 1939). The term "N potential" was introduced by Bancaud in 1953. It indicates that the most important reaction appears at the vertex. The different components of the N potential have a relatively constant latency so that they distinguish themselves clearly from the EEG background noise with an averaging technique. Davis (1965) assumes the following characteristic latencies: P<sub>1</sub> 50–60 s, N<sub>1</sub> 93–100 s, P<sub>2</sub> 170–200 s, N<sub>2</sub> 200 s. In some of the curves these 4 peaks can clearly be distinguished but not always. Most constant is the descending slope of N<sub>1</sub> to P<sub>2</sub>.

#### METHOD

We tried to determine the influence of three stimulus parameters (random on periodically administered stimulus, intensity and frequency of the stimulus tone) on the obtained objective cortical reaction of subjects with a fully normal tonal audiogram. All experiments took place in two acoustically and electrically shielded rooms. In the first, the relaxed subject was lying on a seat with a headphone and electrodes applied to the head.

## CORTICAL AUDIOMETRY IN NORMAL HEARING SUBJECTS

J TYBERGHEIN and G FORREZ

*From the Department of Otolaryngology and the Laboratory of Experimental Otolology and Phoniatrics Academisch Ziekenhuis Sint Rafaël Leuven Belgium*

Our present investigation deals with the influence of three stimulus parameters on slow evoked potentials derived from the intact scalp after acoustic stimulation (sine tones of 60 s duration) in normal hearing subjects. Random application of the stimulus (50 times, every 1 to 3 s) is more efficient than a periodical stimulation (60 times, one stimulus every second). When stimulus intensity increases, the intensity of the reaction—mainly  $N_1$ —also increases, and the latency—mainly  $N_1$  and  $P_1$ —decreases provided that the 40 dB stimulation intensity is not exceeded. The lower frequencies (250 500 1000 and 2000 c/s) give rise to a much clearer cortical reaction than the higher frequencies (4000 and 8000 c/s).

Auditory threshold determination in humans who do not possess the normal introspection possibilities (children or mental-deficients) or do not want to use them (stimulators) has always been a very acute problem.

The first technique of objective audiometry—the psychogalvanic reflex—was described by Féré in 1888. Experience has shown that his results are to be interpreted with reserve. Psychic, visual and tactile stimuli also affect electric skin resistance and can thus cause the disappearance of the response to acoustic stimulation in background noise. Moreover only lesions of the peripheral auditory apparatus, the auditory nerve, the cochlear nuclei and the mesencephalon influence the acoustic psychogalvanic reflex.

During recent years a technique has been discovered that deduces from the intact human scalp the evoked potentials: these arise not only after acoustic but also after tactile and visual stimulation. The cortical reaction has an approximate value of a few microvolts and disappears in the important electric background noise of the scalp (e.g. the EEG) and thus cannot be analysed by means of a simple oscillographic registration. It can however be discovered with special averaging techniques by means of an average response computer because it is time locked to the beginning of the sensorial stimulus. If the sensorial stimulus is repeated a few times, the accidental potential changes of the EEG eliminate each other so that the stimulus locked reaction becomes clearer on the zero line of the EEG.

In all physiological measurements, three factors are to be taken into account: the administered stimulus, the subject, and the registration. As in clinical tonal audiometry the exact determination of the acoustic stimulus

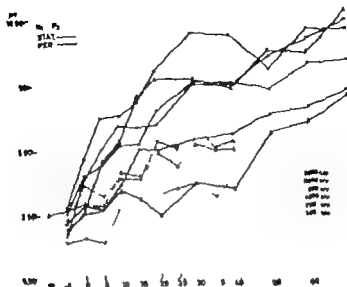


Fig. 3 Comparison of the reaction intensity  $V_{rP}$  (in  $\mu V$ ) in the random (STAT) and the periodical (PER) stimulation series.

(mV meter) a differential amplifier a Data Retrieval Computer and an X-Y-plotter.

The ground electrode was placed on the left ear lobe, one differential electrode was placed 2 cm to the left of the vertex, in an interaural plane, and another on the right ear lobe. These electrodes were connected to a low level amplifier type 122 Tektronix, set at a gain of 1000 with a band pass from 0.8 to 1000 cps. The amplified signal was connected without attenuation to the Data Retrieval Computer Model 7100 (Nuclear Chicago). The signals that were time locked to the stimulus were added, whereas the other signals were averaged. After each series of 50 random or 50 periodical stimuli, the contents of the memory of the Data Retrieval Computer were registered in analog form with an X-Y-plotter model 7590 (AR(S) (Nuclear Chicago). From these graphs we read the extent of the reaction and converted them into an average potential (on the electrodes) for each stimulus.

In both series of measurements the same sound stimuli were used. A pure sine tone generated by a Bruel & Kjaer low frequency generator was connected without any amplitude change to a four diode gate. The gating signal was produced by a monostable multivibrator followed by two integrators, one influencing the rise time and the other the decay time. The time constants had been chosen such that no clicks were to be heard. The envelope of the gating signal is given in Fig. 1a. The duration up to 50% of the curve was 60  $\mu s$ . In the periodical series the monostable multivibrator was triggered by the Data Retrieval Computer itself at the beginning of every analysis period. The analysis time was one second. In the random series separate trigger pulses were generated. They had random intervals

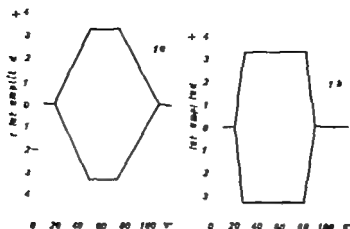
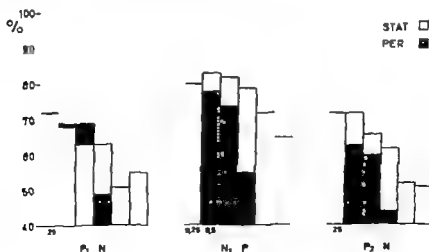


FIG. 1 Shape of the stimulus used.

In the second room were placed stimulation and registration apparatus. In a first periodical series we used sine waves (duration 60  $\sigma$ ) of 500 1000 and 2000 cps at -10 -5 0 5 10 15 20 25 30 35 and 40 dB sensation levels on 81 subjects (42 men and 39 women from ages 10 to 70). The acoustic stimulus was presented sixty times every second. In the second—random—series we used sine waves (duration 60  $\sigma$ ) of 250 500 1000 2000 4000 and 8000 cps at -5 0 5 10 15 20 30 40 50 60 and 70 dB sensation levels on 101 subjects (47 men and 54 women from ages 14 to 63). The acoustic stimulus was presented fifty times with random intervals from 1 to 3". In both experimental series the several frequency intensity combinations were used in a random order. No more than 18 points were measured on a single subject. Every measurement was repeated on 25 different subjects, in order to have statistically reliable results and to neutralize partially the considerable physiological dispersion.

The apparatus used consisted of three electrodes (silver discs of 10 mm



F = Comparison of the mean percentage of correct responses (PER) and statistical significance (STAT) for the three conditions (P1 N, N1 P, P2 N) at the five different intensities (-5, 0, 5, 10, 15, 20, 30, and 40 dB) and the five different frequencies (0.25, 0.5, 1, 2, 4, 8 kc) in the random (STAT) and the periodical (PER) stimulation series.

TABLE 1 Statistical analysis of the results in the random stimulation series

Value of  $t$  where the latency ( $P \setminus P \setminus P \setminus P$ ) is statistically shorter 40 dB than 0, 10, and 20 dB stimulation intensity; value of  $t$  where the latency ( $P \setminus P \setminus P \setminus P$ ) is statistically longer on 40 dB than on 70 dB stimulation intensity; value of  $t$  where the reaction ( $P \setminus P \setminus P \setminus P$ ) is statistically greater on 40 dB than on 0, 10, and 20 dB stimulation intensity; also  $t$  where the reaction ( $P \setminus P \setminus P \setminus P$ ) is statistically smaller 40 dB than on 70 dB stimulation intensity. Test 2% error.  $t$  in italics  $P < 0.01$  Ordinary type  $0.01 < P < 0.05$ .

40 dB	220 $\mu$ , dB				500 $\mu$ , dB				1000 $\mu$ , dB			
	0	10	20	70	0	10	20	70	0	10	20	70
$P$	—	12.08	—	—	17.32	5.86	—	—	4.72	—	—	—
$\setminus$	22.11	12.71	8.61	14.8	11.03	19.11	7.79	—	26.16	7.66	—	—
$P$	22.49	6.09	4.70	—	22.66	17.33	4.11	4.91	16.12	4.52	—	—
$\setminus$	4.54	—	—	—	5.95	—	—	—	12.21	—	—	—
$P \setminus$	5.93	—	—	16.88	11.49	—	—	—	5.52	—	—	14.72
$\setminus P$	27.21	5.28	4.82	11.12	20.22	4.47	—	—	26.61	6.65	—	11.10
$P \setminus$	16.99	—	4.05	—	9.11	—	—	—	16.37	—	—	8.18
40 dB	2000 $\mu$ , dB				4000 $\mu$ , dB				6000 $\mu$ , dB			
	0	10	20	70	0	10	20	70	0	10	20	70
$P$	3.72	—	—	—	—	—	—	—	—	—	—	—
$\setminus$	26.16	11.87	—	—	22.22	8.46	—	—	—	—	—	—
$P$	21.46	16.03	—	—	17.79	5.21	—	—	—	—	—	—
$\setminus$	17.99	—	—	—	9.83	4.21	—	—	—	—	—	—
$P \setminus$	—	—	—	5.60	—	—	—	6.16	—	—	—	37.23
$\setminus P$	16.23	4.89	—	—	17.22	5.10	—	4.19	—	—	—	21.86
$P \setminus$	17.22	—	—	—	5.01	—	—	—	—	—	—	10.22

more frequently in the random than in the periodical series. Moreover the cortical reaction in the random series is much more important than in the periodical series—this appears in Fig. 3, which shows the reaction intensity ( $\mu\lambda$ ) of  $\setminus P_2$  as a function of the stimulus intensity for both the periodical and the random series. Our results confirm the statements of Heldel and collaborators, who have frequently drawn attention to the better reaction to random stimulation than to periodical stimulation. In our experiments this difference is most striking on 2000 cps. On the other hand, the latency has no relation to the way of administering the stimulus, periodical or random (Fig. 4).

Table 1 gives a statistical analysis of the results obtained from the random series. For each of the six frequencies analysed, it is calculated whether there is a significant difference between the latencies  $P_1 \setminus P_2$  and  $\setminus P_2$  on 40 dB and on 0, 10, 20 and 70 dB, and further between the intensities  $P \setminus \setminus P_2$  and  $P_2 \setminus$  on 40 dB and on 0, 10, 20 and 70 dB. Both the latency and the intensity of the reaction appear to be much more dependent upon the stimulus intensity on the low than on the high frequencies; this

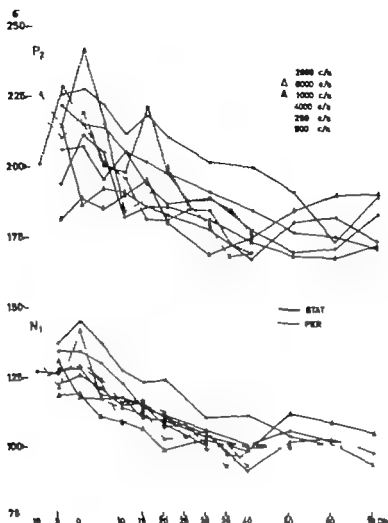


FIG. 4 Comparison of the intensity of  $N_1$  and  $P_2$  (in dB) in the random (STAT) and the periodical (PER) stimulation series

from 1 to 3". These pulses triggered the stimulus generator and the Data Retrieval Computer. The pulsed sine waves thus produced were now further amplified up to a reference level measured by a vacuum tube voltmeter. A manual control was provided to open the gate and give continuous sine waves for calibration. Thereafter followed a calibrated attenuator and a power amplifier to which a headphone (Beyer DT 48) was connected.

## RESULT

Most investigators take the value of  $N_1 P_2$  as a standard for the intensity of the reaction: it is the highest and most frequent peak, as appears from Fig. 2, which represents the average percentages of positive reactions of the three peaks on -5, 0, 5, 10, 15, 20, 30, and 40 dB for the different frequencies. As each point was measured 25 times, we are dealing with average results of 200 ( $8 \times 25$ ) registrations. From Fig. 2 it appears also that the three peaks, with the minor exception of  $P_1 N_1$  on 500 and 1000 cps, occur

normal. La stimulation acoustique à intervalles irréguliers (50 stimulations à ec un intervalle variable (1" à 3") est plus efficiente que la stimulation régulière (50 stimulation avec un intervalle de 1") L'intensité de la réaction (principalement  $N_1$ -P<sub>1</sub>) augmente et le temps de latence (principalement  $N_1$  et P<sub>1</sub> pour autant que les 40 db d'intensité stimuloire ne soient pas dépassés) diminue en fonction de l'intensité de la stimulation acoustique Les fréquences importantes pour la compréhension de la parole humaine (200 500 1000 et 2000 c/s) donnent une réaction corticale beaucoup plus intense que les fréquences aigües (4000 et 8000 c)

## ZUSAMMENFASSUNG

Wir haben den Einfluss von drei Faktoren auf die durch akustische Stimulation (Sinustöne  $N_1$  n 60 s) hervorgerufene Hirnpotentiale beim normalen Menschen geprüft. Die akustische Reizung in unregelmässigen Intervallen (50 Stimuli mit einem unterschiedlichen Intervall von 1 bis 3 Sekunden) ist wirksamer als die regelmässige Reizung (50 Stimuli mit einem Intervall von einer Sekunde). Die Intensität der Reaktion (besonders  $N_1$ -P<sub>1</sub>) nimmt zu, und die Latenzzeit (besonders  $N_1$  und P<sub>1</sub> sofern die Reizintensität von 40 dB nicht überschritten wird) nimmt in Abhängigkeit von der Intensität des akustischen Reizes ab. Die für den menschlichen Sprachbereich wichtigen Frequenzen (200 500, 1000 und 2000 Hz) erzeugen eine viel stärkere kortikale Reaktion als die hohen Frequenzen (4000 und 8000 Hz).

## REFERENCES

- BICKFORD, R. G. JACOBSON, J. L., and COOT, D. T. R., 1964. Nature of evoked potentials (sound and other stimuli). *man. Ann. N.Y. Acad. Sci.* 112 204.
- BONSAVITI, S. J. 1964. Some aspects of auditory evoked potentials. *man. Ann. Otol.* 73 81.
- COOT, D. T. R. JACOBSON, J. L., WILSON, J. C., and BICKFORD, R. G. 1964. A graded evoked myogenic and cortical potential (sound). *man. Ann. Otol.* 73 82.
- COOT, D. T. R. ALLEN, D. W. and BICKFORD, R. G., 1967. Cortical audiometry: A objective method of evaluating auditory acuity in awake and sleeping man. *Trans Amer. Acad. Optol. Otolaryng.* 72 81.
- DALL, H., 1965. Slow cortical responses evoked by acoustic stimuli. *Act. Otolaryng.* (Stockh.) 59 179.
- DALL, H. DALL, P. A. LOONIS, A. L., HANSEN, E. N. and HONERT, G. 1963. Electrical reaction of the human brain to auditory stimulation during sleep. *J. Neurophysiol.* 2, 500.
- GOLDSTEIN, H. and PARRY, L. L., 1966. Clinical use of EEA with evoked response computer: A case report. *J. Speech & Hear. Disord.* 31 75.
- KIDDEL, W. D. and SPRUELL, M., 1965. A diometric aspects and an lithenbory power function of electrically evoked slow evoked cortical responses. *man. Acta Otolaryng.* (Stockh.) 59 201.
- MCCARDY, G. A., and BERT, L., 1966. Summed evoked response to a pure tone stimulus. *J. Speech Res.* 3 204.
- MART, T. E., 1965. Short latency evoked responses to clicks. *J. Appl. Physiol.* 20 725.
- MART, L. SCHIMMEL, H. TOLIN, L. M. KRAUSOV, X. L., and POLLACK, C., 1966. Evoked responses to clicks and tones of varying intensity: waking and sleep. *Elect. Neurophysiol.* 21 325.
- RE, R. M. 1965. Über die Abhängigkeit der objektiv ermittelten Intensitätsfunktion des menschlichen Gehörs von der Tonfolgefrequenz. *Arch. Oh. Nas. K. Rh. Nk. Hk.* 190 122.



is clearly illustrated by the difference between the results on 250 and 8000 cps. As to the latencies, we notice that on the 24 points examined for each peak there is a statistically significant difference 5 times for  $P_1$ , 13 times for  $\lambda_1$ , 13 times for  $P_2$ , and 6 times for  $\lambda_2$ . The latencies  $V_1$  and  $P_2$  appear to be the most stimulus intensity locked. For those two peaks we notice a statistically significant difference 10 times between 10 and 40 dB and only twice between 40 and 70 dB for the 12 examined points. Above the 40 dB stimulus intensity the latency remains almost constant (Fig. 4). Table 1 shows for  $P_1$ ,  $\lambda_1$ , 8 times,  $\lambda_1$ ,  $P_2$ , 15 times, and for  $P_2$ ,  $\lambda_2$ , 11 times a statistically significant difference on the 24 points examined for each peak. The intensity of  $V_1$ ,  $P_1$  thus appears to be most closely related to the stimulus intensity.

Besides the stimulus intensity the frequency of the stimulus has an influence on the reaction intensity. The best reaction is found in the speech area, as appears from Fig. 3 for  $V_1$ ,  $P_2$ . On 4000 and 8000 cps the reaction is considerably lower than on 250, 500, 1000 and 2000 cps—the statistical analysis of the results completely confirms this statement.

To avoid any doubt about the influence of the shape of the stimulus on the change in latency as a function of the intensity, 25 control measurements have been carried out at 3 intensities: 10, 40 and 70 dB and at 6 frequencies: 250, 500, 1000, 2000, 4000 and 8000 cps (450 points). All the measured latencies are about 12  $\sigma$  smaller whereas the rise time of the stimulus has been decreased from 30  $\sigma$  to 8  $\sigma$  (Fig. 1 b).

### DISCUSSION AND CONCLUSIONS

As expected random stimulation gave a bigger reaction than periodical stimulation. There was no other difference observable in the evoked potentials: the latencies were the same and also the relation between the intensity of the stimulus and the amplitude of the potentials. For that reason the major part of our work was done with random stimulation.

A change of the latencies as a function of the stimulus intensity was also expected and was found only for intensities < 40 dB of the sensation level and for the  $\lambda_1$  and  $P_2$  peaks. There was no change in latency for stimuli above 40 dB of the sensation level and the latencies of the  $P_1$  and  $\lambda_2$  peaks seemed to remain almost constant.

Amongst the evoked potential peaks, the total amplitude  $V_1$ ,  $P_2$  was most closely related to the stimulus intensity and seemed to be most suitable to study the slow evoked potentials after acoustic stimulation.

An unexpected result was the following: although the sensation level was always taken as a reference for the acoustic stimuli, the amplitude of the evoked potentials was higher for frequencies in the speech area. A more intensive study of this phenomenon seems to be indicated.

### RÉSUMÉ

Nous étudions l'influence de trois facteurs sur les potentiels évoqués cérébraux dérivés après stimulation acoustique (tons sinusoïdaux de 60 ) chez l'individu

normal. La stimulation acoustique à intervalles irréguliers (50 stimulations avec un intervalle variable de 1 à 3") est plus efficace que la stimulation régulière (60 stimulations avec un intervalle de 1"). L'intensité de la réaction (principalement  $N_1P$ ) augmente et le temps de latence (principalement  $N$  et  $P$ , pour autant que les 40 db d'intensité stimulatoire ne soient pas dépassés) diminue en fonction de l'intensité de la stimulation acoustique. Les fréquences importantes pour la compréhension de la parole humaine (250-500-1000 et 2000 c/s) donnent une réaction corticale beaucoup plus intense que les fréquences basses (1000 et 8000 c).

## ZUSAMMENFASSUNG

Wir haben den Einfluss von drei Faktoren auf die durch akustische Stimulation (Stimulöse von 60) hervorgerufenen Hirnpotential beim normalen Menschen geprüft. Die akustische Reizung in unregelmäßigen Intervallen (50 Stimuli mit einem unterschiedlichen Intervall von 1 bis 3 Sekunden) ist wirksamer als die regelmäßige Reizung (60 Stimuli mit einem Intervall von einer Sekund). Die Intensität der Reaktion (besonders  $N_1P$ ) nimmt zu, und die Latenzzeit (besonders  $N$  und  $P$  sofern eine Reizintensität von 40 dB nicht überschritten wird) nimmt in Abhängigkeit von der Intensität des akustischen Reizes ab. Die für den menschlichen Sprachbereich wichtigen Frequenzen (250, 500, 1000 und 2000 Hz) erzeugen in viel stärkere kortikale Reaktion als die hohen Frequenzen (4000 und 8000 Hz).

## REFERENCES

- BACHFORD, R. H., JACOBSON, J. L., and COOT, D. T. R. 1964, Nature of evoked potentials: sound and their stimuli in man. *Ann. N. Y. Acad. Sci.*, **112**, 361.
- BORSA, L. S. J. 1964, Some aspects of auditory evoked potentials. *Ann. N. Y. Acad. Sci.*, **112**, 361.
- COOT, D. T. R., JACOBSON, J. L., WALLER, J. C., and BACHFORD, R. G. 1964, A staged evoked response and cortical potentials: sound in man. *Ann. N. Y. Acad. Sci.*, **112**, 361.
- COOT, D. T. R., HARRIS, E. W., and BACHFORD, R. G. 1967, Cortical audiometry: A objective method of evoked auditory stimuli in the sleep state. *Trans. Am. Acad. Otolaryng.*, **71**, 81.
- DE LU, R. 1963, Brain cortical responses evoked by acoustic stimuli. *Acta Otolaryng.* (Stockh.), **59**, 179.
- DE LU, R., DE LU, P. A., LOONAN, A. L., HARRIS, E. W., and HOSMER, G. 1965, Electrical reactions of the human brain to auditory stimulation during sleep. *J. Neurophysiol.*, **28**, 508.
- GOLDSTEIN, R. and FRANK, L. L. 1966, Clinical use of EPR with evoked response computer: A case report. *J. Speech Hear. Disord.*, **31**, 71.
- HARRIS, E. W. and SPERDUE, M. 1963, Audiometric peaks and multivocality power in evoked response: A method of evoked low evoked cortical responses. *Ann. N. Y. Acad. Sci.*, **112**, 361.
- MCCAVERTY, G. A. and BART, L. 1964, Stimulated evoked response along parietal stimuli. *J. Appl. Phys.*, **35**, 268.
- MAST, T. E. 1963, Short-latency evoked responses. *Electroencephalogr. Clin. Neurophysiol.*, **20**, 723.
- RAJAN, J., SCHWARTZ, H., TOTTA, L. M., KRASNOV, V. A., and POLLACK, C. 1964, Evoked responses: A method of evoked response: A method of evoked response. *Electroencephalogr. Clin. Neurophysiol.*, **21**, 233.
- RAJAN, H. M. 1963, Über die Abhängigkeit der objektiven ermittelten Intensitätsfunktion des menschlichen Gehörs von der Tonfrequenz. *Arch. Ohr. Nas. Kehlkopfheilk.*, **190**, 133.

- RUBIN, H. WALKER, E., Jr and FLAVIGNY H. 1967 Acoustically-evoked potentials in man. Mediation of early components *Laryngoscope* 77 806
- SPRENG, M. and KENZEL, W. D. 1967 Separierung von Cerebroaudiogramm (CAG) Neuroaudiogramm (NAG) und Otoaudiogramm (OAG) in der objektiven Audiometrie *Arch. Ohr Nas Kehlkopfheilk* 189 225
- STANGE, G. and SODA, T. 1968: Quantitative Untersuchung zur Hörschwelle, Adaptation und Lautheitsempfindung am gesunden und hörgeschädigten Menschen mittels der objektiven Audiometrie *Arch. Ohr Nas Kehlkopfheilk* 190 210
- STUCKI, T. and TAGUCHI, K. 1963 Cerebral evoked response to auditory stimuli in waking man. *Ann. Otol.* 74 128.
- THURKING, J. 1967 Über Beziehungen von Computeraudiogrammen und konventionellen Audiogrammen *Z. Laryng. Rhinol. Otol.* 46 453

Dept. of Otolaryngology  
Acad. Misch. Ziekenhuis Sint Rijk  
Leuven, Belgium

Received May 15 1968

# THE DIAGNOSTIC SIGNIFICANCE OF SPONTANEOUS NYSTAGMUS AS OBSERVED IN THE ELECTRONYSTAGMOGRAPHIC EXAMINATION

A. C. COATS

*From the Department of Physiology Section of Neurophysiology Baylor University College of Medicine and The Methodist Hospital, Houston Tex., U.S.A.*

A study of spontaneous nystagmus in 121 normal subjects and 1601 patients suggests that an "idiopathic spontaneous nystagmus" is recorded with the subject eyes closed in 20-25% of both the normal and patient population. This nystagmus is probably of no significance in the diagnosis of vertigo-producing disorders. It is of low intensity (usually below 6°/sec but occasionally in the 6-10°/sec range) and is more frequently directed to the left than to the right. Spontaneous nystagmus above 10°/sec is of diagnostic significance. It is probable that such a nystagmus will be of peripheral origin and directed away from the side of the peripheral lesion.

Visual fixation is a powerful suppressant of vestibular nystagmus, and therefore recording with electronystagmography (ENG) in the absence of visual fixation very often permits observation of a spontaneous nystagmus which cannot be seen with the patient's eyes open in the light (Aschan *et al.* 1958).

However in the literature on electronystagmography there is considerable controversy as to the diagnostic significance of spontaneous nystagmus once it is recorded. In contrast, the older literature dealing with spontaneous nystagmus present with eyes open is generally in agreement that spontaneous nystagmus is always pathologic and, when caused by an acute peripheral vestibular deficit, is directed away from the side of the deficit (Fischer 1956; Alpers, 1958).

A systematic study of spontaneous nystagmus as recorded electronystagmographically with a goal of redefining the diagnostic significance of this finding, would appear to be desirable. The results of such a study are presented. Emphasis is placed on the significance of the intensity and the direction of the recorded spontaneous nystagmus.

In this report we will consider only "vestibular" spontaneous nystagmus, direction of the recorded spontaneous nystagmus, preserved by visual fixation. We have not considered "central" or "ocular" nystagmus (cf. Dix *et al.* 1963) defined as a nystagmus which is enhanced or unchanged in intensity by visual fixation or which is of different form or direction with eyes closed in comparison to eyes open.

Supported in part by Grant No. 330 from the National Aeronautics and Space Administration.

## MATERIAL AND METHOD

### *ENG Examination Procedure*

#### *General*

All of the normal subjects and patients included in this study were given electronystagmographic examinations at The Methodist Hospital. This examination consisted of a 20° calibration, a test for gaze nystagmus, a position test according to the procedure suggested by Nylén (1939) and later by Aschan *et al* (1956), an optokinetic test and a Fitzgerald Hallpike caloric test. The caloric responses were quantitated by determining maximum eye speed and unilateral weakness and directional preponderance were calculated as a percentage of the total response intensity. Additional details of the test procedure are published elsewhere (Coats, 1965, 1966).

#### *Recording parameters*

Eye movements were recorded on an Offner type R Dynograph with an input time constant of one second and a gain of 100  $\mu\text{V}/\text{cm}$  pen deflection. At this gain eye-movement calibrations ranged from 0.5 to 2°/mm.

#### *Criteria for determining the presence or absence of spontaneous nystagmus*

In order to be considered significant, a spontaneous nystagmus was required to be present, uninterrupted, for at least 30 seconds while the patient was supine during the position test. Also it had to be present in the caloric test position before the start of the first irrigation.

#### *Measurement of spontaneous nystagmus intensity*

Spontaneous-nystagmus intensity was determined by averaging the slow component speeds of at least 5 beats judged to be representative of the average spontaneous-nystagmus intensity. All records were measured by a technician who had no knowledge of the purpose of the study.

### *Normal Subjects*

ENG examinations were given to 121 normal subjects (42 females and 79 males) selected from medical, dental and nursing school students and medical center technical personnel. Ages ranged from 18 to 30 years. All subjects had normal caloric tests and none complained of vertigo or hearing loss.

### *Patients*

Records of 1783 consecutive patients seen at the ENG laboratory in The Methodist Hospital were reviewed. 182 patients were excluded from the study for the reasons listed in Table 1.

The remaining 1601 patients were divided into three major groups, as shown in Table 1. The criteria for each of these groups were as follows:

TABLE 1 Classification of patients

Group		
I	Unilateral weakness	266
II	Other noncentral (D.P. S.N., pos., normal)	1188
III	Evidence of central abnormality	
	A. Evidence other than OPh-gaze NY5	60
	B. Only indication is OPh or gaze NY5	14
	C. CNS abnormality with unilateral weakness	36
Total included in study		1601
Patients excluded from study		
	Bilateral caloric response	42
	Not classifiable	
	No clinical information	14
	Incomplete caloric test	81
	Mastoidectomy	12
	Total not classifiable	107
	Poor records	10
	Ocular or "central" spontaneous nystagmus	31
	Other ocular abnormality	2
Total excluded from study		182
Total — all patients		1,419

*Group I Unilateral weakness*

In this category were placed all patients with caloric responses from one labyrinth which differed from the responses from the opposite labyrinth by more than 20% of the total response. Presumably most patients in this group had unilateral peripheral vestibular lesions.

*Group II All other noncentral*

In this category were placed all of the remaining patients whose ENG and neurological findings did not permit diagnosis of a central nervous system abnormality. Undoubtedly this group includes a large variety of non-vestibular causes of dizziness as well as abnormalities of the peripheral vestibular apparatus which do not produce caloric unilateral weakness (e.g., lesions involving the eighth organs but sparing the semicircular canals).

*Group III Evidence of central nervous system abnormality*

All patients with one or more of the following findings were placed in this group: (1) an optokinetic asymmetry and/or unilateral gaze nystagmus not attributable to a spontaneous nystagmus (Coats, 1963); (2) bilateral gaze nystagmus with eye deviations of 20° from the midline; (3) caloric nystagmus intensely with eyes open and fixed equal to or greater than

## MATERIAL AND METHOD

### *ENG Examination Procedure*

#### *General*

All of the normal subjects and patients included in this study were given electronystagmographic examinations at The Methodist Hospital. This examination consisted of a 20° calibration, a test for gaze nystagmus, a position test according to the procedure suggested by Nylén (1939) and later by Aschan *et al* (1956), an optokinetic test, and a Fitzgerald Hallpike caloric test. The caloric responses were quantitated by determining maximum eye speed, and unilateral weakness and directional preponderance were calculated as a percentage of the total response intensity. Additional details of the test procedure are published elsewhere (Coats, 1965-1966).

#### *Recording parameters*

Eye movements were recorded on an Offner type R Dynograph with an input time constant of one second and a gain of 100  $\mu\text{V}/\text{cm}$  pen deflection. At this gain, eye movement calibrations ranged from 0.5 to 2°/mm.

#### *Criteria for determining the presence or absence of spontaneous nystagmus*

In order to be considered significant, a spontaneous nystagmus was required to be present uninterrupted for at least 30 seconds while the patient was supine during the position test. Also, it had to be present in the caloric test position before the start of the first irrigation.

#### *Measurement of spontaneous nystagmus intensity*

Spontaneous-nystagmus intensity was determined by averaging the slow component speeds of at least 5 beats judged to be representative of the average spontaneous-nystagmus intensity. All records were measured by a technician who had no knowledge of the purpose of the study.

### *Normal Subjects*

ENG examinations were given to 121 normal subjects (42 females and 79 males) selected from medical, dental, and nursing school students and medical center technical personnel. Ages ranged from 18 to 30 years. All subjects had normal caloric tests and none complained of vertigo or hearing loss.

### *Patients*

Records of 1783 consecutive patients seen at the ENG laboratory in The Methodist Hospital were reviewed. 182 patients were excluded from the study for the reasons listed in Table 1.

The remaining 1601 patients were divided into three major groups, as shown in Table 1. The criteria for each of these groups were as follows:

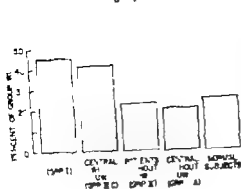


FIG. 1

FIG. 1 Incidence of spontaneous nystagmus in the patient groups listed in Table 1 (group designations shown in parentheses) and in 121 normal subjects.

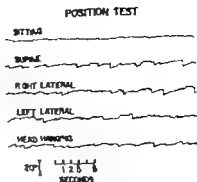


FIG. 2

FIG. 2 Example of spontaneous nystagmus in normal subject. The subject was a 21-year-old right-handed male medical student who had had at 17 years of age 3 weeks before the examination. He had had no other illness, major or minor for at least 6 months and had never had otitis-prudens illness. There was history of ingestion of alcohol or other drugs except for 2 cups of coffee per day. The subject did not smoke. The tympanic membranes were clear. Audiometric and caloric test results were normal. With eyes closed, low intensity left beating spontaneous nystagmus was present in all but the sitting position.

### Intensity of spontaneous nystagmus

Frequency distribution of spontaneous-nystagmus intensity in each of the subject groups are shown in Figs. 3 and 4. In all groups, the most frequently occurring intensity was 3-5/sec (the modal value). However, the unilateral-weakness group differed from the other groups in that there were many more patients in the higher intensity ranges. To facilitate further study of this difference, spontaneous-nystagmus intensity was divided into three ranges: "low" (0.1-5.9/sec), "moderate" (6.0-9.9/sec), and "high" (greater than 9.9/sec). Two (1.6%) of the "normal" subjects had a spontaneous nystagmus in the high intensity range. This is in essential agreement with the results of Bos *et al.* (1963) who found that 3% of their normal subjects had spontaneous nystagmus with an intensity greater than 7.0/sec.

Fig. 5 shows a comparison of the incidence of spontaneous nystagmus in the three intensity ranges. In all three ranges, the incidence of spontaneous nystagmus among patients with unilateral weakness is higher than among patients without unilateral weakness. However, this difference becomes progressively greater as spontaneous-nystagmus intensity increases. The percentages in the three groups without unilateral weakness (central, noncentral, and normal) do not differ significantly.

### Direction of spontaneous nystagmus

The relative incidence of left and right-beating spontaneous nystagmus in the different groups is shown in Fig. 6. It is apparent that in the low



caloric nystagmus intensity with eyes closed (failure of fixation suppression" of caloric nystagmus) (4) definite clinical evidence of a central nervous system abnormality.

Group III was subdivided as shown in Table 1. Since one of the goals of this study was the comparison of the incidence and intensity of spontaneous nystagmus in the presence of central and peripheral pathology, those patients with both central and peripheral pathology (most had mass lesions in the cerebellopontine angle) were grouped separately (Group III C). Fourteen patients in whom the only indication of a central abnormality was an optokinetic asymmetry or gaze nystagmus not attributable to a spontaneous nystagmus (Group III B) were excluded from consideration in order to avoid the use of a criterion based on spontaneous nystagmus to select a group of patients in whom the incidence and intensity of spontaneous nystagmus was to be studied.

The 69 patients in Group III A constitute the CNS abnormality group of primary interest in this study. In 54 of these patients, the presence of CNS pathology was confirmed by evidence other than that provided by the ENG examination. Patients in this group had central lesions of widely varying location and type.

## RESULT

### *Incidence of spontaneous nystagmus*

The percentage of subjects in each group who had a spontaneous nystagmus in the supine position is shown in Fig. 1. Almost half of the patients with caloric unilateral weakness had a spontaneous nystagmus, compared to only 20-25% in the other groups.

The incidence of spontaneous nystagmus in normals is in essential agreement with the results of Bos *et al* (1963) who found nystagmus in one or more positions in 24% of their normal subjects, and of Bergstedt (1961) who found positional nystagmus in 9 of 26 normal subjects. However, it is not in agreement with the statements of other investigators (e.g. Stahle, 1958) that a spontaneous nystagmus observed with eyes closed is always an abnormal finding. A typical example of a spontaneous nystagmus observed in a normal subject is shown in Fig. 2.

It should be emphasized that the recordings on which this report is based were obtained with eyes closed, and not with eyes open in darkness. Dix *et al* (1963) reported that spontaneous nystagmus of central or ocular origin may differ in form or direction when the eyes are closed in comparison to eyes open in darkness. Preliminary results of a study of the nature of spontaneous nystagmus in normals show that opening the eyes in darkness tends to reduce the intensity of the spontaneous nystagmus but never alters its form or direction. The preliminary study also shows that spontaneous nystagmus in normals adds to caloric nystagmus to produce directional preponderance just as it does in patients (Coats, 1966).

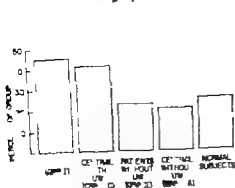


FIG. 1

FIG. 1 Incidence of spontaneous nystagmus in the patient groups listed in Table 1 (group designations shown in parentheses) and in 121 normal subjects.

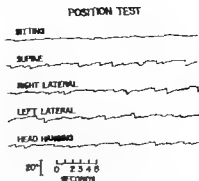


FIG. 2

FIG. 2 Example of spontaneous nystagmus in a normal subject. The subject was 23-year-old right-handed male medical student who had had stuffy nose 2 weeks before the examination. He had had no other illness, major or minor, in the last 6 months and had never had vertigo-producing illness. There was no history of ingestion of alcohol or other drugs except for 2 cups of coffee per day. The subject did not smoke. The tympanic membranes were clear and all metric and caloric tests results were normal. With eyes closed, low-intensity left-beating spontaneous nystagmus was present in all but the sitting position.

### Intensity of spontaneous nystagmus

Frequency distributions of spontaneous-nystagmus intensity in each of the subject groups are shown in Figs. 3 and 4. In all groups, the most frequently occurring intensity was 3.5/sec (the modal value). However the unilateral-weakness group differed from the other groups in that there were many more patients in the higher intensity ranges. To facilitate further study of this difference, spontaneous-nystagmus intensity was divided into three ranges: "low" (0.1-5.9/sec), "moderate" (6.0-9.9/sec) and "high" (greater than 9.9/sec). Two (1.8%) of the "normal" subjects had a spontaneous nystagmus in the high-intensity range. This is in essential agreement with the results of Bos *et al.* (1963) who found that 3% of their normal subjects had spontaneous nystagmus with an intensity greater than 10/sec.

Fig. 5 shows a comparison of the incidence of spontaneous nystagmus in the three intensity ranges. In all three ranges, the incidence of spontaneous nystagmus among patients with unilateral weakness is higher than among patients without unilateral weakness. However this difference becomes progressively greater as spontaneous-nystagmus intensity increases. The percentages in the three groups without unilateral weakness (central, noncentral and normal) do not differ significantly.

### Direction of spontaneous nystagmus

The relative incidence of left and right-beating spontaneous nystagmus in the different groups is shown in Fig. 6. It is apparent that in the low

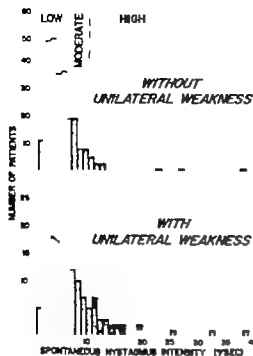


FIG 3

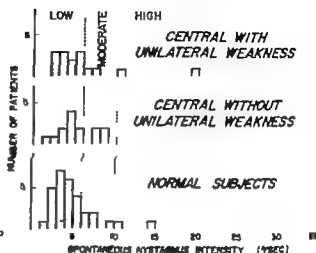


FIG 4

FIG 3 Frequency distribution of spontaneous nystagmus intensity in noncentral patients with (Group I) and without (Group II) caloric unilateral weakness. Because of the much larger number of patients with unilateral weakness, the scale interval on the vertical axis was doubled.

FIG 4 Frequency distribution of spontaneous nystagmus intensity in central patients (Group III A and C) and normal subjects.

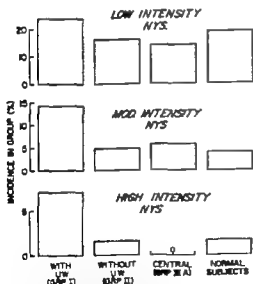


FIG 5

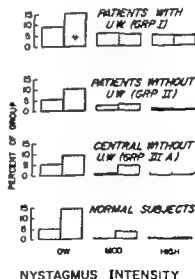


FIG 6

FIG 5 Incidence of pontoon nystagmus of low, moderate and high intensity. The figure compares the relative incidence of spontaneous nystagmus in the unilateral weakness group of equal intensity.

FIG 6 Incidence of right (stippled bars) and left beating (cross-hatched bars) pontoon nystagmus of the three intensity levels in patient groups and normal subjects.

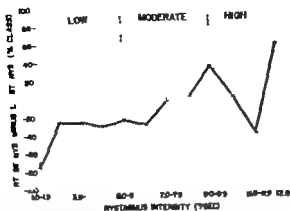


FIG. 7 Effect of intensity on predominance of left over right-beating pontaneous nystagmus. All concentric patients (Groups I and II) with pontaneous nystagmus were separated into intensity classes of 10/sec intervals (horizontal coordinate). The values plotted on the vertical axis were obtained by expressing the difference between numbers of patients with right and left-beating nystagmus as percentage of all patients falling into the intensity interval. The mean predominance of left over right-beating pontaneous nystagmus. Large fluctuations occur at the higher intensity levels because relatively small numbers of patients fell into these intensity classes.

Intensity range, left-beating spontaneous nystagmus predominates in all groups. In the moderate-intensity range, left beating spontaneous nystagmus predominates in the central and normal groups but not in the unilateral weakness and non-unilateral-weakness patient groups. In the high-intensity range the incidence of right and left beating spontaneous nystagmus is about equal in all groups.

In Fig. 7 the relative predominance of right or left beating nystagmus over each 1 intensity increment in Groups I and II is plotted against intensity. In every class below 7.0 /sec, left beating nystagmus predominates. Above 7.0 /sec, incidence of left and right beating-spontaneous nystagmus is essentially equal. The limits of the moderate-intensity range were chosen so that the intensity at which right versus left incidence became equal (7.0-7.9 /sec) would lie equally between them. It is of interest that the intensity at which right-left incidence becomes equal is close to the 6-7 sec "threshold of pathological nystagmus" proposed by Bos *et al* (1963).

In both incidence and predominance of direction to the left, low intensity spontaneous nystagmus in the unilateral weakness group closely resembles low-intensity spontaneous nystagmus in the three non-unilateral weakness groups. Therefore, it would seem probable that, frequently low intensity spontaneous nystagmus, when associated with a unilateral weakness, is not related to the unilateral weakness. This was supported by a comparison of the direction of spontaneous nystagmus relative to the side of the unilateral weakness in the three intensity ranges. The results are shown in

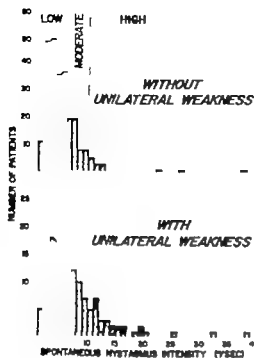


FIG 3

FIG 3 Frequency distribution of spontaneous nystagmus in noncentral patient with (Group I) and with ut (Group II) central unilateral weakness. Because of the much larger number of patients with ut unilateral weakness, the scale is trial on the vertical axis was doubled.

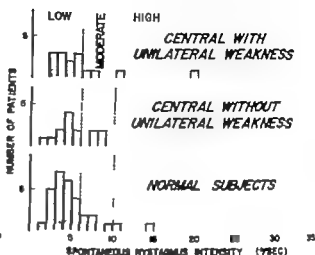


FIG 4

FIG 4 Frequency distribution of spontaneous nystagmus intensity in central patient (Group III A and C) and normal subjects.

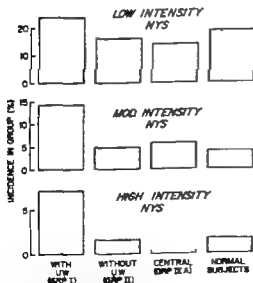


FIG 5

FIG 5 Incidence of spontaneous nystagmus of low, moderate, and high intensity. To facilitate comparison, the relative scale was adjusted to make the plotted incidence of the unilateral weakness group of equal height.

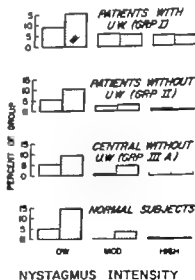


FIG 6

FIG 6 Incidence of right (left) beating spontaneous nystagmus in the three central levels in patient groups and normal subjects.

*Direction of spontaneous nystagmus in patients with caloric unilateral weakness*

Stable (1938) reported that the spontaneous nystagmus was directed away from the affected side in 11 of 13 patients with both "diminished [caloric] sensitivity and spontaneous nystagmus in the caloric test position. In contrast, Jongkees & Philippszoon (1964) reported that the direction of spontaneous nystagmus is poorly correlated with the side of a peripheral vestibular lesion and, therefore, concluded that the direction of spontaneous nystagmus is of no significance. The present study shows that the results of Jongkees & Philippszoon (1964) are obtained only if the intensity of the nystagmus is not considered. If one considers only spontaneous nystagmus of moderate and high intensity, a good relationship between the direction of the spontaneous nystagmus and the side of the peripheral lesion is found.

*Spontaneous nystagmus in patients with central nervous system pathology*

It is of particular interest that the spontaneous nystagmus found in the central group could not in any way be distinguished from idiopathic spontaneous nystagmus occurring in the normal group (it occurred in less than 25% of the patients, was predominantly left-beating, and was exclusively of low or moderate intensity). Because of the broad definition of "central" this result does not exclude the possibility that specific lesions within the central nervous system might produce a vestibular type spontaneous nystagmus. However, that no such lesion was demonstrated among 1601 patients would suggest that such a lesion is uncommon. Therefore a spontaneous nystagmus above 70 /sec is probably an indication of a peripheral vestibular lesion.

It will be recalled that we have excluded from consideration nystagmus which, either with eyes open or closed, did not have a saw-toothed form or which was not suppressed by visual fixation. In our experience central lesions which are generally thought of as producing spontaneous nystagmus (e.g., lesions of the flocculonodular lobe of the cerebellum and multiple sclerosis involving the brainstem) produce nystagmus with one of these characteristics.

## ZUSAMMENFASSUNG

Die Untersuchung eines spontanen Nystagmus bei 121 gesunden Personen und 1601 Patienten ergibt, dass sich bei 20-25% der sowohl gesunden Personen als auch der Patienten ein idiopathischer pontiner Nystagmus bei geschlossenen Augen zeigt. Der Nystagmus ist wahrscheinlich bei der Diagnose schwererer zugrundeliegender Störungen von kleiner Bedeutung. Er ist von geringer Intensität (gewöhnlich unter 6 /Sek. manchmal jedoch in der 6-10 /Sek. Zone) und geht häufig geradlinig oder leicht rechts. Spontaner Nystagmus über 10 /Sek. ist von diagnostischer Bedeutung. Er ist sehr wahrscheinlich, dass solche ein Nystagmus von zentraler Natur ist und von der Seite der zentralen Läsion abhängt.

TABLE 2 *Direction of spontaneous nystagmus relative to side of caloric unilateral weakness (Group I with spontaneous nystagmus)*

Nystagmus intensity	Direction relative to side of U W	Number of patients	% all patients in intensity class
Low	Away	52	50.1
	Toward	36	
Moderate	Away	41	87.2
	Toward	6	
High	Away	25	83.3
	Toward	5	

Table 2 Low intensity nystagmus was directed away from the side of the weakness (the expected direction) in only 50% of the Group I patients with spontaneous nystagmus. However in the moderate and high intensity ranges, this increased to more than 80%.

## DISCUSSION

### *Definition of idiopathic spontaneous nystagmus*

We have found a spontaneous nystagmus in a rather large percentage of subjects presenting themselves as normal. It seems highly unlikely that this nystagmus is of significance in the diagnosis of vertigo-producing disorders. However because of the extreme sensitivity achieved by recording nystagmus in the absence of visual fixation and the impossibility of excluding minor abnormalities from a group of "normal" individuals, we would hesitate to call this a "normal" spontaneous nystagmus. Therefore, the term "idiopathic spontaneous nystagmus" used by Lansberg (1962) is preferred.

On the basis of this survey, the following characteristics of idiopathic spontaneous nystagmus can be defined: (1) It is present with eyes closed in about 20% of people with no evident neuro-otological abnormality. (2) Its speed of slow component is usually less than 8°/sec but may be as high as 9-10°/sec. (3) It usually beats to the left but may be right beating.

### *Idiopathic spontaneous nystagmus in patients*

All of the results of this study support the hypothesis that idiopathic spontaneous nystagmus occurred in patients referred to the ENG laboratory as frequently as it occurred in the normal subjects. This hypothesis would explain the predominance of left beating spontaneous nystagmus in the low intensity range in all subjects, regardless of clinical classification. It would also explain the relatively small increase in incidence of low intensity spontaneous nystagmus among patients with unilateral weakness, and also the poor correlation between side of unilateral weakness and direction of low intensity spontaneous nystagmus in this group.

### *Direction of spontaneous nystagmus in patients with caloric unilateral weakness*

Stable (1938) reported that the spontaneous nystagmus was directed away from the affected side in 11 of 13 patients with both "diminished [caloric] sensitivity" and spontaneous nystagmus in the caloric test position. In contrast, Jongkees & Philippon (1964) reported that the direction of spontaneous nystagmus is poorly correlated with the side of a peripheral vestibular lesion and, therefore, concluded that the direction of spontaneous nystagmus is of no significance. The present study shows that the results of Jongkees & Philippon (1964) are obtained only if the intensity of the nystagmus is not considered. If one considers only spontaneous nystagmus of moderate and high intensity a good relationship between the direction of the spontaneous nystagmus and the side of the peripheral lesion is found.

### *Spontaneous nystagmus in patients with central nervous system pathology*

It is of particular interest that the spontaneous nystagmus found in the central group could not in any way be distinguished from idiopathic spontaneous nystagmus occurring in the normal group (it occurred in less than 2% of the patients, was predominantly left-beating, and was exclusively of low or moderate intensity). Because of the broad definition of "central" this result does not exclude the possibility that specific lesions within the central nervous system might produce a vestibular-type spontaneous nystagmus. However, that no such lesion was demonstrated among 1601 patients would suggest that such a lesion is uncommon. Therefore a spontaneous nystagmus above 7.0 /sec is probably an indication of a peripheral vestibular lesion.

It will be recalled that we have excluded from consideration nystagmus which, either with eyes open or closed, did not have a saw-toothed form which was not suppressed by visual fixation. In our experience central lesions which are generally thought of as producing spontaneous nystagmus (e.g. lesions of the flocculonodular lobe of the cerebellum and multiple sclerosis involving the brain stem) produce nystagmus with one of these characteristics.

### ZUSAMMENFASSUNG

Die Untersuchung des pontinen Nystagmus bei 121 gesunden Personen und 1601 Patienten zeigt, dass er bei 20-23% der sowohl gesunden Personen als auch der Patienten ein idiopathischer spontaner Nystagmus bei geschlossenen Augen ist. Der Nystagmus ist wahrscheinlich bei der Diagnose schwindelerzeugender Störungen von keiner Bedeutung. Er ist von geringer Intensität (gewöhnlich unter 6 Sek., manchmal jedoch in der 6-10 Sek. Zone) und geht häufiger nach links als nach rechts. Spontaner Nystagmus über 10 /Sek. ist von diagnostischer Bedeutung. Es ist sehr wahrscheinlich, dass solche ein Nystagmus von peripherer Entstehung ist und dass der Seit der äußeren Lesion wegführt.



## REFERENCES

- ALPERT, B J 1958 *Vertigo and Deafness* Grune & Stratton, New York.
- ASCHAN G BERGSTEDT M and STAHL, J 1956 Nystagmography Recording of nystagmus in clinical neuro-otological examinations. *Acta Otolaryng* (Stockh.) Suppl. 129
- BERGSTEDT M, 1961 Studies of positional nystagmus in the human centrifuge *Acta Otolaryng* (Stockh.) Suppl. 163
- BOS, J H OOSTERVELD, W J PHILIPAZOON A J WOZZA, J and ZUKA, S., 1963 On pathological spontaneous and positional nystagmus *Pract Otorhinolaryng* (Basel) 25 282.
- COATS, A C., 1965: Electronystagmographic examination History technique, and interpretation *Med Rec Ann* (Houston) 58 48
- 1966: Directional preponderance and spontaneous nystagmus as observed in the electronystagmographic examination *Ann Otol* 75 1135
- 1968: Central and peripheral optokinetic asymmetry *Ann Otol* In press.
- DEY, M R HALLPIER, C S and HOOD J O 1963 Electronystagmography and its uses in the study of spontaneous nystagmus. *Trans Ophthal Soc U K* 83 531
- FISCHER, H J 1956 *The Labyrinth* Grune & Stratton, New York.
- JOHANNES, L B and PHILIPAZOON, A J 1964 Electronystagmography *Acta Otolaryng* (Stockh.) Suppl. 189
- LANZBERG, M P 1962 Latenter idiopathischer Nystagmus *Verhandl T Genesek* 196 393.
- NYLÉN, C O 1939 The oto-neurological diagnosis of tumors of the brain *Acta Otolaryng* (Stockh.) Suppl. 33
- STAHL, J 1958 Electro-nystagmography in the caloric and rotatory tests. *Acta Otolaryng* (Stockh.) Suppl. 137

Department of Physiology Section of  
Neurophysiology Baylor University  
College of Medicine, 1200 Monroney  
Avenue Houston Texas 77025, U.S.A

Received May 27 1968

## CALORIC TESTS IN PASSIVE VASCULAR CONGESTION OF THE LABYRINTH

### *An Electroneystagmographic Study*

S. K. DAS and Y. N. MEHRA

*From the Department of Otolaryngology Postgraduate Institute of Medical Education and Research Chandigarh India*

In order to evaluate the effect of passive venous congestion of the labyrinth on caloric induced nystagmus, thirty normal subjects were given caloric tests in the "head up" and "head down" positions. The horizontal semicircular canal was kept in a vertical position with the ampullated end upward when the head was in the down position and venous congestion was produced. The resulting nystagmus was recorded by an electroneystagmograph. A comparison of the response of the labyrinth in the "head up" and "head down" positions reveals that the response is diminished in the "head down" position more so with cold irrigation. Thus it has been shown that passive venous congestion produces a effect on caloric induced nystagmus.

The response of the labyrinth to caloric stimulation is known to be influenced by various factors, e.g. position and posture of the subject, temperature of the water used for irrigation central inhibition and repellitive caloric stimulation, amongst others.

McNally *et al* (1948) performed caloric tests in supine and prone (face down) positions and found the response to be greater in the supine position than in the prone position in a majority of the cases.

Mehra & Moudgil (1967) observed that response to hot and cold caloric stimulation in the supine position is very significantly higher than in the prone position however they kept the head below the heart level and used electroneystagmographic recording for the study. They put forward the possibility of a passive venous congestion of the labyrinth affecting the caloric response.

Jongkees (1948) studied vascular influence on caloric response. He observed that vasodilation of blood vessels had an effect on the duration of calorically induced nystagmus, and also that such effect appeared slight during hot caloric stimulation and strong during cold caloric stimulation.

The aim of the present study was to evaluate the influence of passive vascular congestion of the labyrinth on the caloric response. Vascular congestion was produced by raising the foot end of the table so as to bring the subject's ear below the heart level while still maintaining the lateral

## REFERENCES

- ALPERT, B. J. 1938 *Vertigo and Dizziness* Grune & Stratton, New York.
- ASCHAN, G. BERGSTEDT, M., and STAHLK, J. 1956 Nystagmography: Recording of nystagmus in clinical neuro-otological examinations *Acta Otolaryng* (Stockh.) Suppl. 129.
- BERGSTEDT, M. 1961: Studies of positional nystagmus in the human centrifuge *Acta Otolaryng* (Stockh.) Suppl. 165.
- BOT, J. H., OOSTERVELD, W. J., PHILIPAZOON, A. J., WOZZA, J. and ZELLA, S. 1963 On pathological spontaneous and positional nystagmus. *Pract. Otorhinolaryng* (Basel) 25: 282.
- COATS, A. C., 1963 Electronystagmographic examination: History, technique and interpretation. *Med. Rec. Ann.* (Houston) 58: 48.
- 1966 Directional preponderance and spontaneous nystagmus as observed in the electronystagmographic examination. *Ann. Otol.* 75: 1135.
- 1966 Central and peripheral optokinetic asymmetry. *Ann. Otol.* In press.
- DRY, M. R., HALLPIKE, C. S. and HOOD, J. O. 1963: Electronystagmography and its uses in the study of spontaneous nystagmus. *Trans. Ophthal. Soc. U.K.* 43: 531.
- FISCHER, B. J. 1956: *The Labyrinth* Grune & Stratton, New York.
- JONGKEER, L. B. and PHILIPAZOON, A. J. 1964 Electronystagmography. *Acta Otolaryng* (Stockh.) Suppl. 189.
- LAUBER, M. P. 1962: Latenter idiopathischer Nystagmus. *Verh. T. Genesek* 166: 396.
- NILÉN, C. O. 1939 The oto-neurological diagnosis of tumors of the brain. *Acta Otolaryng* (Stockh.) Suppl. 33.
- STAHLK, J. 1938 Electro-nystagmography in the caloric and rotatory tests. *Acta Otolaryng* (Stockh.) Suppl. 13.

Department of Physiology, Section of  
Neurophysiology, Baylor University  
College of Medicine, 1200 Moursund  
Avenue, Houston, Texas 77025, U.S.A.

Received May 27 1968

TABLE I

		Cold caloric stimulation				Hot caloric stimulation			
		LCU*	LCD	RCU	RCD	LHU	LHD	RHU	RHD
LP	Mean	19.40	20.03	19.28	21.35	23.11	26.00	24.35	27.88
	S.D.	4.9	5.2	4.5	5.0	4.6	8.3	4.4	8.3
TD	Mean	183.00	180.00	169.06	187.03	184.73	144.93	155.10	144.43
	S.D.	24.6	34.1	35.4	31.2	36.5	36.4	23.2	19.7
TB	Mean	225.03	175.10	222.40	160.20	147.00	127.70	137.06	129.30
	S.D.	50.0	37.0	63.7	33.6	46.1	45.6	38.3	53.2
TA	Mean	1816.13	1199.06	1635.43	1131.16	881.73	748.23	821.40	875.30
	S.D.	677.1	699.4	876.2	702.9	353.6	420.6	434.2	290.2
MSS	Mean	18.90	13.84	17.82	13.30	10.28	9.41	9.58	9.32
	S.D.	4.7	6.3	7.0	5.8	4.0	4.4	4.2	4.8

LCU = Left cold up, etc.

### Analysis of data

The following observations of the records were made: Latent period of onset of nystagmus (LP), Total duration of nystagmus (TD), Total number of beats (TB), Total amplitude (TA), and Maximum speed of slow component (MSS). The mean and standard deviation of various indices of the nystagmus of both series of tests is given in Table I.

The latent period and maximum speed of the slow component show very little variation of values. The total amplitude, total number of beats, and total duration show the maximum variation.

The statistical analysis of the results of the first irrigation with the

TABLE 2. Comparison between the cold and hot responses in the "up" position

Significant value of  $t$  at 0.05 probability: 2.00 and at 0.01 probability: 2.68.

		LCU	RCL	LHU	RHL	"t" value
LP	Mean	26.1		47.76		3.3
	S.D.	3.6		6.4		
TD	Mean	339.88		300.83		3.7
	S.D.	43.5		31.4		
TB	Mean	417.49		251.66		8.7
	S.D.	83.6		60.6		
TA	Mean	3231.36		1646.13		6.9
	S.D.	1167.1		501.6		
MSS	Mean	31.81		19.36		8.0
	S.D.	8.4		3.8		



Fig. 1

semicircular canal in a vertical position. Engorgement of the neck veins was taken as the indicator of vascular congestion in the labyrinth.

Alternate hot and cold caloric tests according to the Fitzgerald & Hallpike technique (1942) were performed on thirty young healthy adults in both the normal supine position and the head low supine position (Figs. 1 and 2). The resulting nystagmus was recorded on an electronystagmograph (Galileo type). This had an AC type preamplifier with a long time constant of 4.5 seconds. The test was performed in a semi-darkened sound treated room. After the application of bitemporal pure silver electrodes, calibration was performed by asking the subject to look at alternating light subtended at a 10-degree angle over the eyes. It was repeated after the test. After each stimulation a rest period of 10 minutes was given.

Caloric tests were done in the following order: (1) Left cold up, (2) right cold up, (3) left cold down, (4) right cold down, (5) left hot up, (6) right hot up, (7) left hot down, (8) right hot down.



Fig. 2.

TABLE 5 Comparison of the total "up" position with the "down" of cold and hot irrigation of the right and left ears

Significant value of  $t'$  at 0.05 probability = 2.00 and at 0.01 probability = 2.68.

		LCU + RCU + LHU + RHU	LCD + RCD + LHD + RHD	$t'$ faces
LP	Mean s.d.	85.50 9.3	98.46 13.7	3.3
TD	Mean s.d.	689.81 87.1	608.19 77.9	3.6
TH	Mean s.d.	732.15 102.0	691.30 111.6	1.7
TA	Mean s.	4937.89 1257.6	4953.65 1115.7	2.2
MS	Mean s.	51.67 10.3	46.07 10.8	2.7

hot and cold irrigation statistical analysis of data was done and the following "t" values resulted. The results show that, except for the latent period, there has been a significant decrease of all the values of nystagmus in the "down" position with cold irrigation, whereas it is not significant with hot irrigation.

Comparison of the total "up" responses of the right and left ears with the total "down" position of the right and left ears is given in Table 4. This table again shows that there is a diminished response in the "down" position.

The total "up" position and total "down" position are compared in Table

The result again shows that there is a greater diminution of response in the "down" position than that in the "up" position. Thus it can be summarized that passive venous congestion of the labyrinth may be one of the factors suppressing the response. Somehow this effect is more marked with cold stimulation than with hot stimulation. Jongkees (1948) compared the result of caloric tests in the usual position with those of the "upside down" position. He observed that duration of the nystagmus was shorter in the upside down than the usual position. He has also noted that the effect of vasodilatation of the labyrinth produced with amyl nitrite was less marked with hot caloric stimulation than with cold caloric stimulation. Our finding also seems to correspond with Jongkees. Mehra & Moudgil (1967) also noted a reduction of response in the "face down" position.

It is postulated from this study that passive venous congestion of the labyrinth produces a diminished response to cold caloric stimulation. It is the intention of the authors to pursue this investigation on patients with raised jugular venous pressure.

TABLE 3 *Comparison of  $t$  values between the cold and hot responses in the up and down positions*Significant value of " $t$ " at 0.05 probability = 2.00 and at 0.01 probability = 2.68.

	LCU + LCD	LHU + LHD	RCU + RCD	RHU + RHD
LP	1	1.84	1.7	2.0
TD	2.00	1.20	2.31	1.9
TB	3.62	1.62	3.2	1
TA	2.34	1.20	2.4	1
NSS	2.20	1	2.6	1

second irrigation and comparison of right and left ear sensitivity do not show any significant difference Lidvall (1962) had observed from his studies of repeated caloric stimulation that response decline to repeated stimuli is more pronounced with identical stimuli, but was of the opinion that transfer of habituation probably does not cause distortion of routine caloric tests

To note whether hot and cold stimulations are equal the total cold irrigation response in the "up" position is compared with the total hot irrigation in the "up" position in Table 2. The result indicates that there is a marked reduction of all values of nystagmus except in the latent period responses of hot irrigation. Thus hot stimulation is less effective than cold. Similar findings have been noted by McNally *et al* (1948) and Mehra (1964). However Aschan *et al* (1956) and Stahle (1956) have observed no difference.

To compare individually each ear for the 'up' and "down" positions for

TABLE 4 *Comparison of responses of the "up" position of the left and right ears with those of the down position in the same ears*Significant value of " $t$ " at 0.05 probability = 2.00 and at 0.01 probability = 2.68.

		Left ear		$t$ value	Right ear		$t$ value
		LCU + LHU	LCD + LHD		RCU + RHU	RCD + RHD	
LP	Mean	43.63	49.23	2.2	66.50	96.46	3.3
	S.D.	9.7	9.7		9.3	13.7	
TD	Mean	332.06	301.46	2.2	669.81	606.19	3.6
	S.D.	68.6	36.9		67.1	77.9	
TB	Mean	360.12	298.50	3.0	732.15	601.30	4
	S.D.	73.1	83.2		102.0	111.6	
TA	Mean	2456.83	2005.46	2.2	4937.69	3953.53	3.2
	S.D.	816.1	760.4		1257.6	1115.7	
NSS	Mean	27.40	22.82	2.3	54.67	56.07	2.7
	S.D.	7.7	7.5		10.3	10.8	

# DIE RICHTCHARAKTERISTIK PRIMÄRER AFFERENZEN DES OTOLITHENORGANS BEI INTAKTER EFFERENTER INNERVATION

M. GIESEN und R. KLINKE

*Aus der Hals-Nasen-Ohrenklinik und dem Physiologischen Institut der Freien  
Universität Berlin Berlin Deutschland*

Meerschweinchen konnten auf einem Kardanischen Tisch um alle drei Achsen des Raumes gekippt werden. Von primären Fasern des Nervi vestibularis wurden Aktionspotentiale abgeleitet. Auch bei intakter efferenter Innervation zeigen die primären Fasern eine Funktionsweise wie sie von Löwenstein und Roberts in isolierten Rochenlabirinth gefunden wurde. Die primären Fasern die vermutlich benachbart und daher ähnlich orientierte Rezeptorzellen innervieren, besitzen eine eindimensionale Richtcharakteristik. Daher müssen wenigstens drei verschiedene Rezeptortypen existieren, damit der Organismus eine umkehrbar eindeutige Information über die Lage im Raum gewinnen kann. Tatsächlich konnten verschiedene Rezeptortypen gefunden werden die den von Spoendlin nachgewiesenen verschiedenen Orientierungsrichtungen der Macula-Rezeptoren entsprechen. Die Überbestimmung der Funktionsweise primärer afferenter Fasern sowohl bei intakter als auch bei abgeschalteter efferenter Innervation wirft die Frage der funktionellen Bedeutung der efferenten Innervation auf.

Arbeitsdiagramme von primären Maculaafferenzen wurden bereits 1950 von Löwenstein & Roberts (1950) veröffentlicht. Die Befunde wurden am isolierten Rochenlabirinth gewonnen. Sie zeigen, daß in den primären Fasern eine Spontanaktivität von außerordentlicher Regelmäßigkeit besteht. Auf diese Spontanaktivität wird die Information über die Lage des Tieres im Raum durch Änderung der Entladungsrate aufmoduliert. Die Regelmäßigkeit der Entladungen bleibt dabei bestehen. Die Autoren beschreiben ferner noch sogenannte out of position Neurone und die „into level“ Neurone. Diese Neurone zeigen bei Normalposition des Tieres minimale bzw. maximale Aktivität. Bei Kippung in jeder beliebigen Richtung nimmt die Aktivität zu bzw. ab.

Die Sinnesepithel der Vestibularorgane besteht aus sekundären Sinneszellen. Außer der afferenten Innervation dieser Zellen wurde auch eine efferente Innervation nachgewiesen. Diese Kenntnis verdanken wir elektrophysiologischen Untersuchungen von Wersäll (1956) sowie Engström & Wersäll (1958), histologischen Arbeiten von Ramussen & Gacek (1958) von Gacek (1960) und von Rossi & Cortesina (1962). Folgende liegen neuro-

Mit Unterstützung der Deutschen Forschungsgemeinschaft Gr 161/6 und Gr 161/8



## ZUSAMMENFASSUNG

Um die Wirkung der passiv giftigen Stauung des Labyrinths auf den durch Kalorien herbeigeführten Nystagmus zu berechnen wurden bei dreissig normalen Subjekten kalorische Versuche bei hochgehaltenem und gesenktem Kopf durchgeführt. Der horizontale halbzirkuläre Kanal wurde in vertikaler Stellung — mit aufrechter Ampulle — gehalten wenn der Kopf in gesenkter Stellung war und giftige Stauungen wurden bewirkt. Der erzielte Nystagmus wurde durch den Elektronystagmograph verzeichnet. Ein Vergleich der Reaktionen des Labyrinths bei hochgehaltenem oder gesenktem Kopf hat gezeigt, dass die Wirkung bei gesenktem Kopf geringer ist um so mehr bei kalter Irrigation. Somit zeigte sich, dass passiv giftige Stauungen eine Wirkung auf den durch Kalorien herbeigeführten Nystagmus erzeugen.

## REFERENCES

- ASCHAN, G. BERGSTEDT, M. and STAHL, J. 1956: Nystagmography *Acta Otolaryng* (Stockh.) Suppl. 129.
- FITZGERALD, G. and HALLPIKE, C. S., 1942: Studies in human vestibular function I. Observation in the directional preponderance (Nystagmusbereitschaft) of caloric nystagmus resulting from cerebral lesion *Brain* 65: 115.
- JONGKEER, L. B. W. 1948: Value of caloric test of labyrinth. *Arch. Otolaryng* (Chic.) 48: 402.
- LIDY, L. H. F. 1962: Specific and nonspecific traits of habituation in nystagmus responses to caloric stimuli *Acta Otolaryng* (Stockh.) 55: 315.
- MCCLELLY, W. J., STUART, E. A., JAMIESON, J. S. and GAULTON, C., 1948: Some experiments with caloric stimulation of the human labyrinth to study the relative values of ampullopetal and ampullopetal and lymphatic flow (Ewald's laws) *Trans. Amer. Acad. Ophth. & Otolaryng.* 5: 513.
- MEHRA, Y. N. 1964: Electronystagmography: A study of caloric test in normal subjects. *J. Laryng.* 78: 520.
- MEHRA, Y. N. and MOUDON, B. D. 1967: Evaluation of Ewald's second law—an electronystagmographic study *Acta Otolaryng* (Stockh.) 63: 33.
- STAHL, J. 1956: Electro-nystagmography in the caloric test *Acta. Soc. Med. Upsal* 61: 307.

*Dept. of Otolaryngology Postgraduate  
Institute of Medical Education &  
Research Chandigarh India*

*Received February 28, 1968*

## ERGEBNISSE

Afferente Neurone aus den Maculaorganen zeigen auch bei intakter efferenter Innervation eine spontane Aktivität von außerordentlich hoher Regelmäßigkeit. Wird die Lage des Tieres im Raum geändert, so ändern sich im allgemeinen auch die Entladungsraten. Der neue Wert wird nach einer geringgradigen und wenige Sekunden andauernden phasischen Aktivierung bzw. Hemmung erreicht und konstant eingehalten. Darüber hinaus können auch bei längerer Beobachtungszeit keine weiteren Adaptationserscheinungen gefunden werden. Die Veränderung der Entladungsrates hängt von der Kipprichtung ab. Für jedes Neuron läßt sich eine — und nur eine — Richtung finden, in der das Neuron maximal aktiviert wird. Bei Kippung in die entgegengesetzte Richtung wird das Neuron gehemmt. Diese Richtung maximaler Aktivierung ist für jedes Neuron verschieden.

Die Abbildung 1 zeigt Originalregistrierungen. Bei dem hier gezeigten Neuron war eine maximale Aktivierung bei Kippung nach vorn zu beobachten. In der Mitte ist die Aktivität des Neurons in Normalposition des Tieres dargestellt. Darüber ist die Entladungsfolge bei Hebung des Kopfes um 30° darunter bei Senkung um 30° zu sehen. Die außerordentlich große Regelmäßigkeit der Entladung ist auf den ersten Blick zu erkennen.

Die Aktivität eines Neurons bei verschiedenen Kippstellungen läßt sich in einem Zylinderkoordinatensystem darstellen. Eine solche Darstellung der Aktivität desselben Neurons zeigt Abbildung 2. Wie man sieht, ist jede

Z 52-1-13



Z 52-1-1



Z 52-1-18



05 sec

Abb. 1 Originalregistrierung einer primären Maculaaffäre bei Normalposition (Mitte) Hebung des Kopfes um 30° (oben) und Senkung des Kopfes um 30° (unten)

physiologische Befunde von Schmidt (1963) von Gleisner & Henriksson (1963) sowie von Sala (1965) vor

Über die funktionelle Bedeutung der efferenten Innervation am Macula organ liegen noch keine deutbaren Befunde vor. Rupert *et al* (1962) haben primäre afferente Vestibularisfasern der Katze bei intakter efferenter Innervation untersucht. Sie beschreiben Neurone mit regelmäßigen Entladungen, deren Entladungsrate sich mit der Kippstellung ändert. Komplette Arbeitadiagramme fehlen jedoch. Weitere Untersuchungen an primären Vestibularisfasern der Katze wurden von Wing (1963) veröffentlicht. Er hat bei seinen Versuchen aber das Vorderhirn abgesaugt und die Katzen in Arbeitadiagramme fehlen jedoch. Weitere Untersuchungen an primären efferenten Innervation intakt geblieben ist. Er kam zu dem Ergebnis, daß das Otolithenorgan rudimentär und ohne nennenswerte Funktion sei. Cramer (1962) hat offenbar von Neuronen höherer Ordnung abgeleitet.

Es war das Ziel der hier beschriebenen Versuche, die Beobachtungen von Rupert *et al* (1962) zu ergänzen, das Ergebnis von Wing (1963) zu überprüfen und die von Löwenstein & Roberts (1950) gefundenen Funktionsdiagramme der Maculaafferenzen bei erhaltener efferenter Innervation zu untersuchen.

#### METHODE

Die Untersuchungen wurden an Meerschweinchen durchgeführt. Zwei orientierende Versuche wurden an Katzen vorgenommen. Die Tiere wurden zunächst mit einem kurz wirksamen Thiobarbiturat (Trapanal 30 mg/kg i.p.) narkotisiert. Es erfolgte eine Tracheotomie und die Einlage einer Beatmungskanüle. Die Tiere wurden sodann mit Flaxedil relaxiert und künstlich beatmet. Sämtliche Operationsstellen und alle Druckstellen, die der Kopfhalter verursachte, wurden sorgfältig mit Xylocain lokal anästhesiert. Außer der Anfangsdosis wurde kein Narkotikum nachgegeben. Die Barbituratwirkung war zum Zeitpunkt des Beginns der Registrierung abgeklungen. So konnte eine Depression der narkoseempfindlichen efferenten Aktivität (Gleisner & Henriksson 1963) vermieden werden. Nach Eröffnung der Schädelskalotte über dem Kleinhirn wurden die Tiere auf einem kardanischen Tisch gelagert. Die Körpertemperatur der Tiere wurde durch eine Heizung konstant gehalten.

Der kardanische Tisch, der um alle drei Achsen des Raumes zu kippen war, erlaubte es, das Tier in jede beliebige Position zu bringen. Nach dem stereotaktischen Atlas von Rössner (1965) wurden die auf das Kerngebiet zulaufenden Fasern des Nervus vestibularis mit metallgefüllten Mikropipetten aufgesucht. Die Aktivität dieser Neurone wurde bei verschiedenen Kippstellungen untersucht. Nach jeder Änderung der Kippstellung wurde 60 Sekunden abgewartet, um irgendwelche dynamischen Komponenten mit Sicherheit auszuschließen. Teilweise wurde nach dem Versuch die Lage der Mikroelektrode durch Koagulation markiert. Die Aktionspotentiale wurden mit einem Digitalcomputer (LINC 8) ausgewertet.

# ERGEBNISSE

Afferente Neurone aus den Maculaorganen zeigen auch bei intakter efferter Innervation eine spontane Aktivität von außerordentlich hoher Regelmäßigkeit. Wird die Lage des Tieres im Raum geändert, so ändern sich im allgemeinen auch die Entladungsraten. Der neue Wert wird nach einer geringgradigen und wenige Sekunden andauernden phasischen Aktivierung bzw. Hemmung erreicht und konstant eingehalten. Darüber hinaus können auch bei längerer Beobachtungzeit keine weiteren Adaptationserscheinungen gefunden werden. Die Veränderung der Entladungsrates hängt von der Kipprichtung ab. Für jedes Neuron läßt sich eine — und nur eine — Richtung finden, in der das Neuron maximal aktiviert wird. Bei Kippung in die entgegengesetzte Richtung wird das Neuron gehemmt. Diese Richtung maximaler Aktivierung ist für jedes Neuron verschieden.

Die Abbildung 1 zeigt Originalregistrierungen. Bei dem hier gezeigten Neuron war eine maximale Aktivierung bei Kippung nach vorn zu beobachten. In der Mitte ist die Aktivität des Neurons in Normalposition des Tieres dargestellt. Darüber ist die Entladungsfolge bei Hebung des Kopfes um 30° darunter bei Senkung um 30° zu sehen. Die außerordentlich große Regelmäßigkeit der Entladung ist auf den ersten Blick zu erkennen.

Die Aktivität eines Neurons bei verschiedenen Kippstellungen läßt sich in einem Zylinderkoordinatensystem darstellen. Eine solche Darstellung der Aktivität desselben Neurons zeigt Abbildung 2. Wie man sieht, ist jede

Z52-1 13



Z52-1-1



Z52-1-18



05 sec

Abb. 1 Originalregistrierung einer primären Maculaafferenz bei Normalposition (Mitte), Hebung des Kopfes um 30° (oben) und Senkung des Kopfes um 30° (unten).

physiologische Befunde von Schmidt (1963) von Gleisner & Henriksson (1963) sowie von Sala (1965) vor

Über die funktionelle Bedeutung der efferenten Innervation am Maculaorgan liegen noch keine deutbaren Befunde vor. Rupert *et al* (1962) haben primäre afferente Vestibularisfasern der Katze bei intakter efferenter Innervation untersucht. Sie beschreiben Neurone mit regelmäßigen Entladungen deren Entladungsrate sich mit der Klippstellung ändert. Komplette Arbeitsdiagramme fehlen. Jedoch weitere Untersuchungen an primären Vestibularisfasern der Katze wurden von Wing (1963) veröffentlicht. Er hat bei seinen Versuchen aber das Vorderhirn abgesaugt und die Katzen in Arbeitsdiagramme fehlen. Jedoch weitere Untersuchungen an primären efferenten Innervation intakt geblieben ist. Er kam zu dem Ergebnis, daß das Otolithenorgan rudimentär und ohne nennenswerte Funktion sei. Cramer (1962) hat offenbar von Neuronen höherer Ordnung abgeleitet.

Es war das Ziel der hier beschriebenen Versuche, die Beobachtungen von Rupert *et al* (1962) zu ergänzen, das Ergebnis von Wing (1963) zu überprüfen und die von Löwenstein & Roberts (1960) gefundenen Funktionsdiagramme der Maculaafferenzen bei erhaltener efferenter Innervation zu untersuchen.

#### METHODE

Die Untersuchungen wurden an Meerschweinchen durchgeführt. Zwei orientierende Versuche wurden an Katzen vorgenommen. Die Tiere wurden zunächst mit einem kurz wirksamen Thioharbiturat (Trapanal 30 mg/kg i.p.) narkotisiert. Es erfolgte eine Tracheotomie und die Einlage einer Beatmungskanüle. Die Tiere wurden sodann mit Flaxedil relaxiert und künstlich beatmet. Sämtliche Operationsstellen und alle Druckstellen, die der Kopfhalter verursachte, wurden sorgfältig mit Xylocain lokal anästhesiert. Außer der Anfangsdosis wurde kein Narkotikum nachgegeben. Die Barbituratwirkung war zum Zeitpunkt des Beginns der Registrierungen abgeklungen. So konnte eine Depression der narkoseempfindlichen efferenten Aktivität (Gleisner & Henriksson 1963) vermieden werden. Nach Eröffnung der Schädelkalotte über dem Kleinhirn wurden die Tiere auf einem kardanischem Tisch gelagert. Die Körpertemperatur der Tiere wurde durch eine Heizung konstant gehalten.

Der kardanische Tisch, der um alle drei Achsen des Raumes zu kippen war, erlaubte es, das Tier in jede beliebige Position zu bringen. Nach dem stereotaktischen Atlas von Rossner (1965) wurden die auf das Kerngebiet zulaufenden Fasern des Nervus vestibularis mit metallgefüllten Mikropipetten aufgesucht. Die Aktivität dieser Neurone wurde bei verschiedenen Klippstellungen untersucht. Nach jeder Änderung der Klippstellung wurde 60 Sekunden abgewartet, um irgendwelche dynamischen Komponenten mit Sicherheit auszuschließen. Teilweise wurde nach dem Versuch die Lage der Mikroelektrode durch Koagulation markiert. Die Aktionspotentiale wurden mit einem Digitalcomputer (LINC 8) ausgewertet.

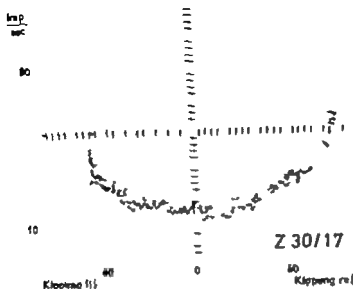


Abb. 2 Entladungsrate eines Neuron mit minimaler Aktivität bei Normalposition. A tonometrische Aktivität mit einem Herrn Klauske gebaute elektronischen Zähler

handelte ihre Aktivität war durch Berührung der homolateralen Kopfsparthe zu steigern. Die Elektrodenspitze lag somit in diesen Fällen nicht im richtigen Gebiet.

Die von uns beobachtete Änderung der Entladungsrate bei Kippung des Tieres ist gut korrelierbar mit den Potentialänderungen des Bestandspotentials im Sinneszellager der Macula utriculi bei künstlicher Abscherung des Oolithen (Trineker 1960, 1961).

## DISKUSSION

In Analogie zu den Hogenängen kann angenommen werden, daß es immer dann zu einer Aktivierung im primären afferenten Neuron kommt, wenn das Zilienbündel der zugehörigen Rezeptorzellen in Richtung des Kinoziliums abgeschert wird. Nun ist die Orientierung der Zellen im Sinneszell erkrank der Maculae nicht einheitlich. Nach polarisationsoptischen Untersuchungen von Spendlin (1961, 1963) können praktisch alle Richtungen des dreidimensionalen Raumes als Orientierungsrichtung auftreten, wenn man die Macula utriculi und die Macula sacculi als funktionelle Einheit zusammenfaßt. Die Abb. 4 zeigt die Orientierung der Kinozilien auf den Maculae nach Befunden von Spendlin (1963). Wenn man annimmt, daß eine afferente Faser jeweils einige benachbarte und damit gleich orientierte Sinneszellen versorgt, dann lassen sich unsere neurophysiologischen Befunde zwanglos den anatomischen Befunden von Spendlin (1963) zuordnen. Nach ihnen sind in der Tat sehr viele verschiedene Richtungen maximaler Akti-

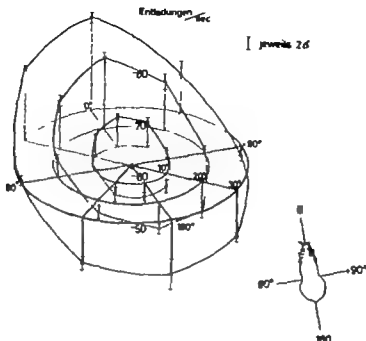


Abb. 2 Arbeitsdiagramm eines primären afferenten Neurons aus dem Maculaorgan eines Meeresschnecken bei Kippung in verschiedene Richtungen

Kippstellung mit einer bestimmten Entladungsrate korreliert, doch ist die Korrelation nicht umkehrbar eindeutig. Bei der Kippung nach halblinks bzw. halbrechts ergeben sich ungefähr gleiche Entladungsraten. Die einzelne afferente Faser besitzt also nur eine eindimensionale Richtcharakteristik, wie sie auch von Gärner (1963) am Sellenlinienorgan von *Xenopus laevis* beschrieben wurde.

Neben den Neuronen, deren Aktivität bei Kippung nach vorn maximal zunahm, konnten, wie schon erwähnt, auch andere Typen gefunden werden, die bei Kippung nach seitlich oder hinten maximal aktiviert wurden. Schließlich wurde auch ein Neuron gefunden, das in der Normalposition des Tieres eine minimale Aktivität zeigte, während andere Neurone zeigten, dass in der Normalposition des Tieres eine maximale Aktivität vorlag. Wurde das Tier aus der Normalposition gekippt, so nahm unabhängig von der Kipprichtung einmal die Aktivität zu, im anderen Fall nahm sie ab. Die Abbildung 3 zeigt ein Neuron mit minimaler Entladungsrate in Normalposition. Beim Kippen sowohl nach rechts als auch nach links nahm die Aktivität zu. Das galt auch für Kippung nach vorn und hinten.

Spontan stumme, also in Normalposition inaktive Neurone wurden nicht beobachtet.

Doppelentladungen, wie sie von Rupert *et al.* (1962) beschrieben wurden, konnten von uns nicht beobachtet werden. Wir fanden zwar Neurone, die Doppelentladungen mit sehr kurzem Intervall aufwiesen, sie zeigten auch eine Abhängigkeit von der Tierlage. Bei genauerer Analyse zeigte sich jedoch immer, daß es sich um Einheiten aus dem Kerngebiet des Trigeminus

Wenstein & Roberts (1950) am isolierten Rochenlabyrinth und unseren Befunden am Meerschweinchen. Sofern die Ergebnisse vom Rochen mit denen am Meerschweinchen überhaupt vergleichbar sind, ist bei statischen Reizen auf den Otolithenapparat kein Einfluß der Efferenz auf die Entladung im afferenten Neuron festzustellen. Es stellt sich also um so mehr die Frage nach der funktionellen Bedeutung der efferenten Innervation.

Innerhalb gewisser Grenzen ist das Vestibularorgan ohne Zweifel in der Lage, Information über die Stellung im dreidimensionalen Raum zu liefern. Ein dreidimensionaler Raum wird durch drei linear unabhängige Vektoren aufgespannt. Wenn die primären Afferenzen — wie aus unseren Untersuchungen hervorgeht — ein eindimensionales Richtcharakteristik besitzen, dann müssen mindestens drei verschiedene Typen von afferenten Fasern bzw. Rezeptoren existieren, die sich in ihrem Arbeitsbereich, nicht aber in ihren prinzipiellen Eigenschaften unterscheiden, und die die drei linear unabhängigen Vektoren repräsentieren. Sowohl nach unseren Untersuchungen als auch nach den Befunden von Spoendlin (1963) ist das der Fall, wenn man Utriculus und Sacculus als funktionelle Einheit auffaßt. Das System ist sogar mathematisch überbestimmt. Im übrigen dürfte der Utriculus die größere biologische Bedeutung besitzen.

Der Ansicht von Wing (1963), daß die Maculaorgane bei der Katze rudimentär und ohne nennenswerte funktionelle Bedeutung seien, vermögen wir uns nicht anzuschließen.

Wir danken Herrn Dr. Dr. O. J. Gröver für die Anregung zu den Untersuchungen sowie für die kräftige Unterstützung der Arbeit. Frau A. Thiele danken wir für die sorgfältige technische Assistenz, Herrn H. Danenberg für den Bau des kardanischen Tisches.

## SUMMARY

Cat pupils were placed on a kardan table which permitted the positioning of the animals in all possible spatial directions. Action potentials of single primary fibers were recorded. Even with intact efferent innervation the fibers show functionally characteristic initials like those described by Löwenstein and Roberts for the isolated labyrinth of the ray. The primary fibers, which are supposed to supply several neighbouring and hence similarly oriented receptors, possess individual directional characteristics. Thus at least three receptor types are required for the unequivocal localization of position in three-dimensional space. A variety of receptor types were actually found which apparently correspond to the morphologically oriented cells of the sensory epithelium of the maculae. The operation characteristics of the primary fibers are the same with and without intact efferent innervation, the influence of the efferent fibers is therefore not apparent.

## LITERATUR

- C. A. H. L., 1962: Response of mammalian organically receptors to sustained tilt. *J. neurophysiol.* 25: 663.  
 F. M. H. and W. H. L., 1954: The structural organization of the organ of Corti and of the external sensory epithelia. *Exp. Cell Res.* Suppl. 3: 140.



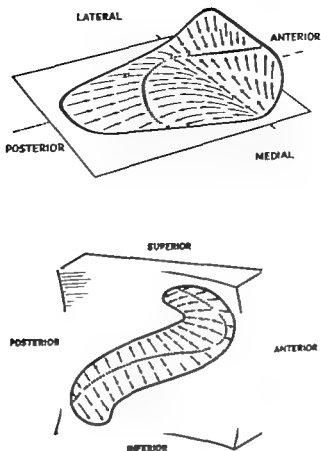


Abb. 4 Orientierung d. Kinocilien auf der Macula utriculi (oben) und der Macula sacculi (unten) nach Spoendlin (1965). Die Pfeile zeigen die Richtung des Kinociliums.

vierung zu erwarten. Auch die von uns gefundenen Neuronentypen, die bei Normalposition des Tieres eine minimale bzw. maximale Aktivität aufweisen, sind nach den Befunden von Spoendlin (1960) zu fordern. Es sind Afferenzen von den Sinneszellen der Macula sacculi, die so orientiert sind, daß ihr Kinocilium bei Normalposition des Tieres ungefähr nach oben bzw. nach unten zeigt.

Selbstverständlich konnte es sich bei den von uns gefundenen Neuronen auch um die von Löwenstein & Roberts (1950) beschriebenen „out of position“ und „into level“ Neurone handeln. Diese zweifellos besonders gearbete Neuronenklasse besteht jedoch möglicherweise aus Afferenzen von der Lagena (MacNaughton & McNally 1946), die beim Sauger fehlt. Wir sind daher der Ansicht, daß die von uns gefundenen bei Normalposition minimal bzw. maximal aktiven Neurone mit solchen Rezeptorzellen verschaltet sind, auf die der Otolith — wahrscheinlich der Sacculusotolith — in Normalposition einen minimalen bzw. maximalen Reiz ausübt, die sich aber sonst ebenso verhalten wie die übrigen Neurone. Diese Hypothese konnte jedoch nicht endgültig überprüft werden, da im Versuch der Kontakt zu diesen Neuronen jedesmal bereits bei einer Klippstellung von  $\pm 60^\circ$  verloren ging. Abgesehen von dieser noch nicht endgültig geklärten Frage besteht jedoch eine auffallende Übereinstimmung zwischen den Befunden von Lo-

Lowenstein & Roberts (1950) am isolierten Rochenlabyrinth und unseren Befunden am Meerschweinchen. Sofern die Ergebnisse vom Rochen mit denen am Meerschweinchen überhaupt vergleichbar sind, ist bei statischen Reizen auf den Otolithenapparat kein Einfluß der Efferenz auf die Entladung im afferenten Neuron festzustellen. Es stellt sich also um so mehr die Frage nach der funktionellen Bedeutung der efferenten Innervation.

Innerhalb gewisser Grenzen ist das Vestibularorgan ohne Zweifel in der Lage, Information über die Stellung im dreidimensionalen Raum zu liefern. Ein dreidimensionaler Raum wird durch drei linear unabhängige Vektoren aufgespannt. Wenn die primären Afferenzen — wie aus unseren Untersuchungen hervorgeht — ein eindimensionale Richtcharakteristik besitzen, dann müssen mindestens drei verschiedene Typen von afferenten Fasern bzw. Rezeptoren existieren, die sich in ihrem Arbeitsbereich, nicht aber in ihren prinzipiellen Eigenschaften unterscheiden, und die die drei linear unabhängigen Vektoren repräsentieren. Sowohl nach unseren Untersuchungen als auch nach den Befunden von Spoendlin (1963) ist das der Fall, wenn man Utriculus und Sacculus als funktionelle Einheit auffaßt. Das System ist sogar mathematisch überbestimmt. Im übrigen dürfte der Utriculus die größere biologische Bedeutung besitzen.

Der Ansicht von Wing (1963), daß die Maculaorgane bei der Katze rudimentär und ohne nennenswerte funktionelle Bedeutung seien, vermögen wir uns nicht anzuschließen.

Wir danken Herrn Priv. Doz. Dr. O. J. Gruber für die Anregung zu den Untersuchungen sowie für die tatkräftige Unterstützung der Arbeit. Frau A. Thiele danken wir für die sorgfältige technische Assistenz, Herrn H. Dannenberg für den Bau des kardaniischen Tisches.

## SUMMARY

Guinea pigs were placed on a cardanic table which permitted the positioning of the animals in all possible spatial directions. Action potential of single primary fibers were recorded. Even with intact efferent innervation the fibers show functional characteristics similar to those described by Lowenstein and Roberts for the isolated labyrinth of the ray. The primary fibers, which are supposed to supply several neighbouring and hence similarly oriented receptive fields, show multidimensional directional characteristics. Thus at least three receptor types are required for the unequivocal location of position in three-dimensional space. Various types of receptors were actually found which apparently correspond to the morphologically oriented cells in the sensory epithelium of the maculae. As the operation characteristics of the primary fibers are the same with and without intact efferent innervation, the influence of the efferent fibers is therefore not yet apparent.

## LITERATUR

- CHAMBERLAIN, R. L., 1962. Response of human Macula gracilis receptors to sustained tilt. *Ann. New York Acad. Sci.* 94: 943.  
 LOWENSTEIN, W. and ROBERTS, J., 1950. The ultrastructural organization of the organ of Corti of the vestibular sensory epithelia. *Exp. Cell Res.* 5: 460.

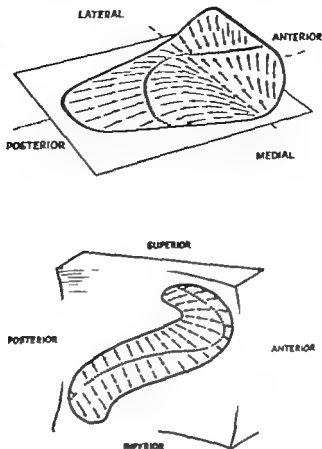


Abb. 4 Orientierung d. Kinocilien auf der Macula utricle (oben) und der Macula sacculi (unten) nach Spoendlin (1965). Der Pfeil weist in Richtung des Kinocilliums.

vierung zu erwarten. Auch die von uns gefundenen Neuronentypen, die bei Normalposition des Tieres eine minimale bzw. maximale Aktivität aufweisen, sind nach den Befunden von Spoendlin (1965) zu fordern. Es sind Afferenzen von den Sinneszellen der Macula sacculi, die so orientiert sind, daß ihr Kinezillum bei Normalposition des Tieres ungefähr nach oben bzw. nach unten zeigt.

Selbstverständlich konnte es sich bei den von uns gefundenen Neuronen auch um die von Lowenstein & Roberts (1950) beschriebenen out of position und into level Neurone handeln. Diese zweifellos besonders gearbete Neuronenklasse besteht jedoch möglicherweise aus Afferenzen von der Lagena (MacNaughton & McNally 1946), die beim Säuger fehlt. Wir sind daher der Ansicht, daß die von uns gefundenen bei Normalposition minimal bzw. maximal aktiven Neurone mit solchen Rezeptorzellen verschaltet sind, auf die der Otolith — wahrscheinlich der Sacculusotolith — in Normalposition einen minimalen bzw. maximalen Reiz ausübt, die sich aber sonst ebenso verhalten wie die übrigen Neurone. Diese Hypothese könnte jedoch nicht endgültig überprüft werden, da im Versuch der Kontakt zu diesen Neuronen jedesmal bereits bei einer Klippstellung von  $+60^\circ$  verloren ging. Abgesehen von dieser noch nicht endgültig geklärten Frage besteht jedoch eine auffallende Übereinstimmung zwischen den Befunden von Lö-

## HEARING LOSS AND CALORIC RESPONSE IN MENIÈRE'S DISEASE

### A Comparative Study

A. ENANDER and J. STAHLÉ

From the Department of Otolaryngology, University Hospital, Uppsala, Sweden

In 343 patients with Menière's disease the caloric response was compared with the hearing loss as measured by pure tone audiometry and a distinct correlation was found. With increasing tone threshold the caloric reaction from the affected ear tended to become weaker and the number of patients with a reduced caloric reaction increased. One hundred and thirty-eight patients underwent speech audiometry and the maximal discrimination was calculated. No correlation was found between discrimination loss and reduction of the caloric reaction. The tracing width of the Békésy audiogram on stimulation with continuous tone was compared with the caloric reaction in 53 patients. A correlation was found. The relationship between the separation between the thresholds for continuous and interrupted tone in Békésy audiometry and the caloric reaction was studied in 40 patients. A distinct correlation was found. The greater the separation between the two Békésy recordings, the greater often was the reduction of the caloric response.

Characteristic of Menière's disease is a disturbance of both the cochlear and the vestibular function. It is not yet established, however, to what extent lesions in the cochlea and labyrinth are from a functional aspect, mutually connected. In two previous studies the caloric reaction (Stahlé & Bergman, 1967) and the pure tone audiogram (Enander & Stahlé, 1967) have been treated separately. It was evident from these investigations that the majority of the patients had an abnormal caloric response but that this response was reduced in only about half of them. All patients had a sensory neural hearing loss, and the mean value for the tone threshold in 334 patients was 52 dB. Among the pure tone audiograms the most common type of curve was the "flat loss type" (60%). The hearing loss seemed to occur mainly during the first year of the disease after which there was only a small increase with time. No special configuration of the pure tone audiogram, pathognomonic for early or late stages of the disease, was found.

Recruitment of loudness, first described by Fowler (1936) is today a well known phenomenon in Menière's disease and other disorders of hearing of a cochlear origin (Dix *et al.*, 1948). In 1947 Békésy showed that in hearing loss due to cochlear lesions the tracing width of the Békésy audio-

- GACEK, R R 1960 Efferent component of the vestibular nerve In *Neuromechanisms of the Auditory and Vestibular Systems* (Ed G L Rasmussen und W F Wiedle) p. 274-284 Springfield, Illinois.
- GLEISNER, L., und HENRIKSSON N G 1963: Efferent and afferent activity pattern in the vestibular nerve of the frog. *Acta Otolaryng* (Stockh.) Suppl 192 90
- GÖRNER, P 1963: Untersuchungen zur Morphologie und Elektrophysiologie des Seitenhöhlenorgans vom Krallenfrosch (*Xenopus laevis* Daudin) *Z Vergleich Physiol* 47 314.
- LÖWENSTEIN, O und ROBERTS, T D M 1950 The equilibrium function of the otolith organs of the thornback ray (*Raja clavata*) *J Physiol* 110 392
- MACNAUGHTON I P J und McNALLY W J 1946: Some experiments which indicate that frogs' lagena has equilibrium function. *J Laryng* 61 204
- RASMUSSEN G L., und GACEK, R R 1958 Concerning the question of an efferent fiber component of the vestibular nerve of the cat *Anat Rec* 130 341
- RÖSNER, W 1963 Stereotaktisch. Atlas vom Meerschweinchen München.
- ROMI, G und CONTESINA, G 1962 Sistema efferent collaterale cocleo-otolare *Minerva Otorinolaring* 12 1
- ROBERT A MOSCHOWITZ G und GALAMBOS, H 1962 Microelectrode studies of primary vestibular neurons in cat *Exp Neurol* 5 100
- SALA, B 1965: The efferent vestibular system Electrophysiological research. *Acta Otolaryng* (Stockh.) Suppl 197
- SCHMIDT R S 1963: Frog labyrinthine efferent impulses. *Acta Otolaryng* (Stockh.) 56 51
- SPOENDLIN H 1964 Organization of the sensory hairs in the gravity receptors in utricle and saccule of the squirrel monkey *Z Zelforsch* 62 701
- 1965 Strukturelle Eigenschaften der vestibulären Rezeptoren. *Schweizer Arch Neurol Psychiat* 96 219
- TRINCKER, D 1959 Neuere Untersuchungen zur Elektrophysiologie des Vestibular-Apparates *Die Naturwissenschaften* 46 344
- 1967: Persönliche Mitteilung
- VERMALL, J 1956: Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig *Acta Otolaryng* (Stockh.) Suppl 126
- WILSON, M E., 1963 The response of the otolith organ to tilt *Acta Otolaryng* (Stockh.) 56 537

H M Giesen HVO Klinik der  
Friedrich-Wilhelms-Universität Bonn  
Spandauer Damm 130

Dr R Klinkke Physilogisches Institut der  
Freien Universität Berlin Arnim II 22  
D-1 Berlin 33 Deutschland

Eingegangen am 16. Mai 1968

Hz, always in the direction from low to high frequency. The interruption rule for the interrupted tracing was 2.5 l.p.s.

For speech discrimination tests the Swedish PB word lists were used. The level of presentation was 30 dB above the speech reception threshold. When the discrimination could not be determined at that level because of discomfort the discrimination tests were presented at three to four different levels and an articulation curve was plotted.

### Caloric testing

The caloric tests were performed with water at 30 C. and 44 C. and in cases where no reaction was obtained with these stimuli, ice water was used. The patients lay in the horizontal supine position with the head raised 30° (Stahlé 1956, Stahlé & Sahl 1964). The reactions were recorded by means of electronystagmography (Aschan *et al.* 1956).

The caloric responses were assessed exclusively on the basis of the eye speed in the slow nystagmus phase which is a considerably more sensitive indicator of labyrinthine function than the duration of the response (Henriksson, 1956, Stahlé 1956, 1958, 1968). The mean eye speed during a 10-second period at the peak of the reaction was assessed and denoted "maximum intensity". As a measure of the caloric response the percentage difference between the maximum intensities from the right and left ear was calculated, according to principles given by Jongkees & Philippon (1964). The normal limit for this difference was placed at 20% (=2.5 times the standard deviation).

### MATERIAL

A total of 343 patients were investigated, and all of these exhibited the typical triad of symptoms. The average duration of the disease was 8 years. The series included 227 patients who had had such severe vertigo that after the examination they underwent an operation (ultrasonic irradiation). All patients were tested with pure tone audiometry and electronystagmography by uniform methods.

For studies of Békésy audiograms, those patients were selected for whom recordings had been made with a Grason-Stadler E-800 audiometer. Since this apparatus had been available for only a short time this series was limited. The tracing width was calculated for 53 patients, and the separation between the recordings of continuous and interrupted tone was studied in 40 patients.

Speech discrimination, finally, had been measured in 138 of the patients.

### RESULTS

#### *Pure Tone Hearing Loss and Caloric Response*

The hearing loss in 343 patients was correlated with the difference between the air and bone conduction reactions from the two labyrinths. The hearing loss was calculated from the mean value of the air conduction at frequencies of 500, 1000, and 2000 Hz. The mean hearing loss for the entire series was

gram was narrowed in the frequency region for which the patient showed recruitment. This observation has since been supported by Lundborg (1959) who investigated 20 patients with Menière's disease and found that all of them exhibited narrowing of the tracing width. Békésy considered that narrowing of this width constituted indirect proof of recruitment. This relationship has been questioned however by Hirsh *et al* (1954) Landes (1958) and Jerger (1962).

With the method introduced by Jerger (1960) in Békésy audiometry in cochlear damage a separation is often found between the recordings of continuous and interrupted tone within the high frequency range. Harbert & Young (1962) have pointed out that early cases of Menière's disease often show normal amplitude and superposition of continuous and interrupted tracings, and late cases show reduced amplitude and separation between interrupted and continuous tracings.

The speech discrimination loss in Menière's disease has been the subject of several studies, and reports in the literature show a discrepancy between the results of different investigators. While some authors (Passe 1953, Lidén 1954) have found that poor discrimination is a typical feature others have found that the discrimination is only moderately or slightly impaired (Opheim & Floitorp 1957, Jerger 1960). An articulation curve of a hump-back type has been considered to be typical of Menière's disease (Cawthorne & Harvey 1953). On comparing the speech discrimination loss and hearing loss for pure tones, Cawthorne & Harvey (1953) found no constant relationship and Goodman (1965) found that they could fluctuate independently of one another.

Studies with light microscopy have shown changes especially in the cochlea and vestibule and to a smaller extent in the semicircular canals, and large individual differences can occur (Kristensen, 1961, Lindsay 1960, 1966, Schuknecht 1963, Allmann & Kornfeld 1965). Our own studies of the function of the inner ear have shown that permanent cochlear functional loss is more common than vestibular functional loss (Stahlé & Bergman 1967). Whether the reason for this is that in certain cases the disease affects the cochlea to a higher degree or that the semicircular canals are more resistant, is not known.

The aim of the investigation reported below was to study retrospectively in a large series of patients, the relationship between loss of cochlear function and loss of function of the semicircular canals.

## METHODS

### *Hearing tests*

The pure tone audiograms were recorded with an Amplivox (Model 61) audiometer and narrow band masking was used. Békésy audiometry was carried out with a Grason Stadler E-800 audiometer. The attenuation rate was 2.5 dB per second. Sweep-frequency tracings for both interrupted and continuous tone stimuli were obtained for the frequency range 125-8000

Hz, always in the direction from low to high frequency. The interruption rate for the interrupted tracing was 2.5 l.p.s.

For speech discrimination tests the Swedish PB word lists were used. The level of presentation was 30 dB above the speech reception threshold. When the discrimination could not be determined at that level because of discomfort the discrimination tests were presented at three to four different levels and an articulation curve was plotted.

#### *Caloric testing*

The caloric tests were performed with water at 30 C and 44 C, and in cases where no reaction was obtained with these stimuli, iced water was used. The patients lay in the horizontal supine position with the head raised 30° (Stahle 1956, Stahle & Sahl 1964). The reactions were recorded by means of electronystagmography (Aschan *et al.* 1956).

The caloric responses were assessed exclusively on the basis of the eye speed in the slow nystagmus phase which is a considerably more sensitive indicator of labyrinthine function than the duration of the response (Henriksson, 1956; Stahle 1956, 1958, 1968). The mean eye speed during a 10-second period at the peak of the reaction was assessed and denoted "maximum intensity". As a measure of the caloric response the percentage difference between the maximum intensities from the right and left ear was calculated, according to principles given by Jongkees & Philipsson (1961). The normal limit for this difference was placed at 20% ( $=2\sigma$  times the standard deviation).

#### MATERIAL

A total of 343 patients were investigated, and all of these exhibited the typical triad of symptoms. The average duration of the disease was 8 years. The series included 227 patients who had had such severe vertigo that after the examination they underwent an operation (ultrasonic irradiation). All patients were tested with pure tone audiometry and electronystagmography by uniform methods.

For studies of Békésy audiograms, those patients were selected for whom recordings had been made with a Grason Stadler E-800 audiometer. Since this apparatus had been available for only a short time this series was limited. The tracing width was calculated for 53 patients, and the separation between the recordings of continuous and interrupted tone was studied in 40 patients.

Speech discrimination, finally, had been measured in 138 of the patients.

#### RESULTS

##### *Pure Tone Hearing Loss and Caloric Response*

The hearing loss in 343 patients was correlated with the difference between the caloric reactions from the two labyrinths. The hearing loss was calculated from the mean value of the air conduction at frequencies of 500, 1000, and 2000 Hz. The mean hearing loss for the entire series was



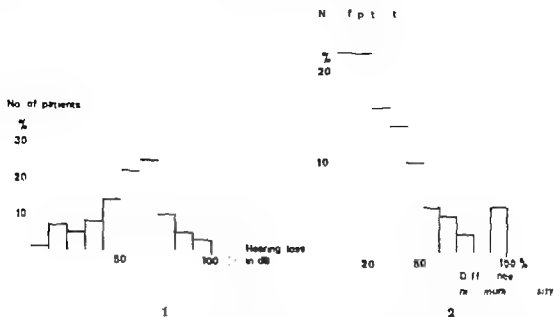


FIG. 1. Percentage distribution of 343 patients with Menière's disease according to hearing loss.

FIG. 2. The results of the caloric test in 343 patients with Menière's disease expressed as the percentage difference in the maximum intensities recorded from the right and left sides. The normal limit for this difference is set at 20%. The percentage distribution of patients is given on the ordinate.

53.4 dB. The distribution of patients according to degree of hearing loss can be seen in Fig. 1. The difference in maximum intensity for the 343 patients was, on the average, 29%; the distribution of patients according to degree of reduction of the caloric response is shown in Fig. 2. The relation between caloric response and hearing loss can be seen in Fig. 3, which is based on a classification of the patients into 5 groups according to degree of hearing loss: 0–10 dB, 28 patients; 20–39 dB, 47 patients; 40–59 dB, 123 patients; 60–79 dB, 110 patients; and 80–100 dB, 26 patients. The mean values for the maximum intensity differences in these 5 groups were 16.5, 18.1, 20.5, 33.7, and 40.5, and these values are plotted in the figure. There was a distinct but weak statistical correlation between the magnitude of the hearing loss recorded and the reduction of the caloric response. The coefficient of correlation was 0.31, which is significant at a significance level of 5%.

In order to elucidate these questions further, the 343 patients were divided into 4 groups according to their degree of hearing loss, and the caloric reaction in the different groups was then studied (Fig. 4a, b, c, and d). Out of 30 patients with a hearing loss not exceeding 20 dB (Fig. 4a) the caloric reaction was normal in 25 and reduced in 5. On the other hand, among 130 patients with a hearing loss of 61 dB and over (Fig. 4d) the caloric reaction was normal in 44 and reduced in 86. Thus, with an increasing degree of hearing loss, the number of patients with a reduced caloric response increased.

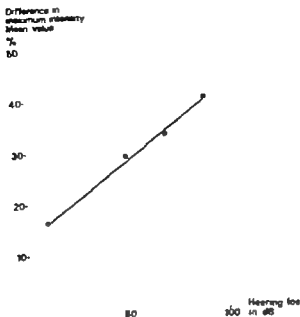


FIG. 3. The relation between hearing loss and caloric response (difference in maximum intensity see text (Fig. 2)) 243 patients with Menière's disease

#### *Maximum Discrimination Score and Caloric Response*

The maximum discrimination score in 138 patients was, on the average 53%. The distribution of patients with different degrees of discrimination loss is shown in Fig. 5. As can be seen in Fig. 6, no constant relationship between the maximum discrimination score and the caloric response was found. Neither was any correlation shown on statistical analysis.

#### *Békésy Audiometry and the Caloric Response*

##### *(a) The tracing width of the Békésy audiogram and the caloric response*

According to Lundborg (1952) the decrease in the tracing width of the continuous tone found in patients with Menière's disease occurs as a rule in the frequency range of 1000 Hz and upwards. We therefore calculated the mean width of the tracing in the frequency range 1000–8000 Hz for each patient. A comparison based on the results from 53 patients is shown in Fig. 7. It is evident from this figure that in patients with a narrow tracing width, both normal and greatly reduced caloric reactions can be seen. No correlation was found on statistical analysis.

Piva (1956) suggested that recruitment should be considered to be present when the tracing width of the continuous tone is less than 5 dB. Of the 53 patients studied, 28 showed a mean width of the tracing of less than 5 dB within the frequency range 1000–8000 Hz, while 25 patients had

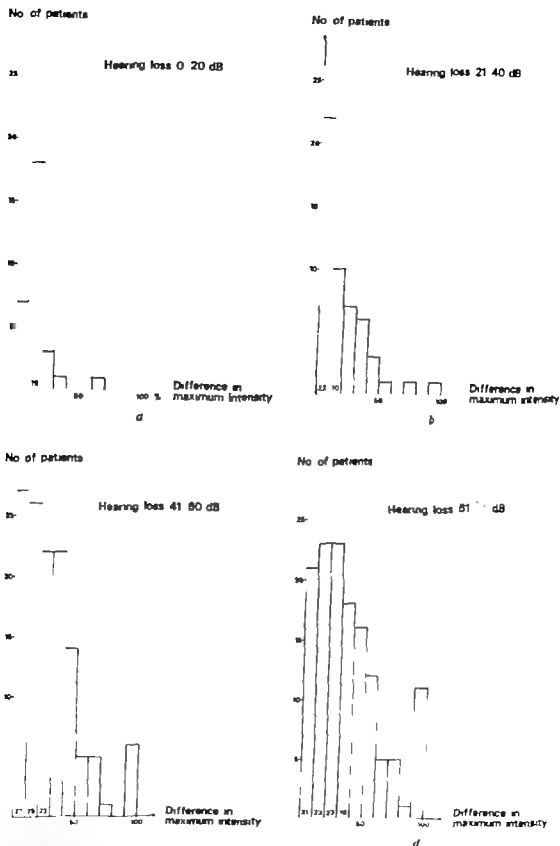


Fig. 4 a-d The loudness response (difference in maximum intensity) according to Fig. 2) in 4 groups of patients with different degrees of hearing loss. With an increasing degree of hearing loss the number of patients with a reduced loudness response tends to increase. The numbers in the columns represent the number of patients.

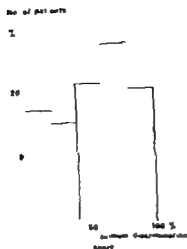


FIG. 5. The percentage distribution of 138 patients according to maximum discrimination score.

a mean width of 5 dB or more. The maximum intensity difference for the former group was 29% and for the latter group 27%. This minor difference was not statistically significant.

*(b) The separation between interrupted and continuous tracings in the Békésy audiogram and the caloric response*

The study of the separation was limited to the frequency range 500–8000 Hz, since Owens (1964) pointed out that in cochlear lesions the threshold

On the left  
Maximum intensity  
%  
00 100

Difference in  
maximum intensity  
%  
0 100



FIG. 6. Correlation between the maximum discrimination score and the caloric response (difference in maximum intensity) in 138 Ménière patients. No indication of any constant relationship between these two factors is shown. The dotted line indicates the normal limit in the caloric response.

FIG. 7. Comparison between the tracing width of the Békésy diagram and the caloric response (ordinal) in 52 patients. No correlation is shown.

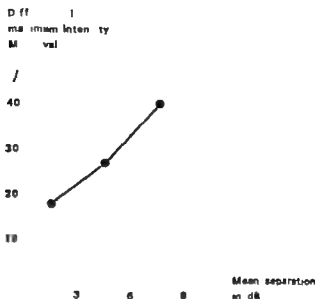


FIG 8 Comparison between the mean separation of interrupted and continuous tracings in Békésy audiogram (abscissa) and the caloric response (ordinate) in 40 patients. A distinct correlation is shown.

shift of the steady tone Békésy tracings was less reliable in frequencies below 500 Hz. The separation was measured from the bottom of the spikes. The mean separation within the frequency range 500–8000 Hz was calculated for each patient and correlated with the maximum intensity difference in a total of 40 patients. As can be seen in Fig 8 there is a clear correlation. Fig 8 was based on the following mean values: Group 0–3 dB, 8 patients with a difference in maximum intensity of 17.8%; 3–6 dB, 16 patients with a difference of 27.0%; 6–9 dB, 16 patients with a difference of 39.8%. Statistical analysis showed a correlation coefficient of 0.27 which means significance at a significance level of 10%. It is concluded that the greater the separation the greater the tendency to a reduction of the caloric response.

#### COMMENTS

Histopathological findings reported in the literature seem to indicate that the semicircular canals are affected by the lesion to a different degree from the cochlea and vestibule. A common finding in studies of recent years is that the disease affects predominantly the membranous walls, while on light microscopy no marked changes have been noted in the neural structures. Distension, bulging, and ruptures of the membranous walls have been shown in the cochlea and vestibule in particular but only to a small extent in the semicircular canals. The functional disturbances are considered to be due both to biochemical alterations in the labyrinthine fluids and to a direct mechanical effect on the sensory end-organs in connection with ruptures (Schuknecht, 1963; Altmann & Kornfeld, 1965). Lindsay (1966) has gone one step further and assumed that this is a question of different mechanisms.

of attack in the cochlea and labyrinth, where the auditory disturbances are due to alterations in the chemical composition of the labyrinthine fluids, while vestibular manifestations are an expression of a direct mechanical effect of the collapsed membranous labyrinth on the vestibular end-organs.

In the light of reports of certain differences in the histopathological picture and different theories as an explanation for functional changes in the cochlea and labyrinth, we considered it of interest to present a clinical study of the relationship between auditory and vestibular function. Our study was based, however, on the results of only a few selected function tests, and the comparison must therefore be regarded as incomplete.

The results of our investigation indicate that there is a relationship between hearing loss, recorded by pure tone audiometry and the caloric response. The greater the increase in the tone threshold, the greater the tendency to reduction of the caloric response and the greater the number of patients with a reduced response. When, on the other hand, the tone threshold was raised to only a minor extent, the caloric reaction was often normal. It should be emphasized that in approximately 40% of the patients the caloric reaction was normal in spite of a hearing loss in all cases. This lack of agreement can probably be explained primarily by the inadequacy of the technique for examination of the semicircular canals. As a secondary explanation, however, the possibility should be considered that in many cases the disease attacks the cochlea to a greater degree than the semicircular canals. This latter theory may have some support in observations at light microscopy.

A weak but distinct relationship was shown, further, between the threshold shift of continuous tone in sweep-frequency Békésy tracings, and the caloric response. Usually the greater the separation between the continuous and the interrupted Békésy tracings, the greater was the reduction of the caloric response. On the other hand, no correlation was found either between the caloric response and the discrimination loss or between the caloric response and the tracing width of the continuous tone in Békésy audiometry.

The reason for the finding that certain hearing tests show a correlation to the caloric response, while others show no such correlation must be that the different hearing tests reflect different aspects of the hearing function. According to Goodman (1965) the hearing level for tones and the discrimination loss for speech in Menière's disease can fluctuate independently of one another. Similar observations have been made previously by Pásse (1953). Goodman considers that these findings "lend support to the possibility that different and probably independent sets of conditions underlie hearing loss for tones and discrimination loss for speech". Huizing & Reynijes (1952) demonstrated a close relationship between discrimination loss and recruitment. The pathological loudness function, i.e. recruitment, tends to distort with consequent discrimination loss. Recruitment is considered generally to be a manifestation of hair cell damage. The reduced amplitude

of the Békésy audiogram has been considered to be an indirect recruitment test, but this has been questioned during recent years. The threshold shift of the continuous Békésy tracings is an expression of adaptation (Jerger 1960). Studies of cochlear microphonics have shown, however, that adaptation is not a phenomenon associated with the hair cells.

In view of the above reports the reduced width of the tracing in Békésy audiometry and also the discrimination loss in speech audiometry could partially reflect hair cell damage. Our investigation showed that there was no significant relationship between the results of those hearing tests in which the changes could largely reflect hair cell damage and the caloric response.

On the other hand we did find a correlation between the caloric response and those hearing tests in which the change in Menière's disease reflected a direct hair cell lesion to only a minor extent but where the lesion lay at another perhaps more central level in the cochlea (pure tone audiogram and separation). With our as yet limited knowledge of the neurophysiology of the inner ear and of the basic cause of the recruitment phenomenon, however, these assumptions can only be considered hypothetical.

### ZUSAMMENFASSUNG

Bei 343 Patienten mit Menièrescher Krankheit wurde die kalorische Reaktion mit dem tonaudiometrisch gemessenen Hörverlust verglichen und ein eindeutiger Zusammenhang konnte festgestellt werden. Je höher die Tonschwelle lag, desto schwächer war meist auch die kalorische Reaktion des kranken Ohrs. Um diesen Zusammenhang deutlicher in Erscheinung zu bringen, wurden die Patienten dem Hörverlust entsprechend in vier Gruppen eingeteilt und die kalorische Reaktion wurde in den verschiedenen Gruppen festgelegt. Unter 30 Patienten mit einer Hörverschlechterung von höchstens 20 dB konnte nur in 5 Fällen eine Herabsetzung der kalorischen Reaktion festgestellt werden. Unter 130 Patienten mit einer Hörverschlechterung von über 60 dB wurde Herabsetzung der kalorischen Reaktion in 91 Fällen nachgewiesen. Mit zunehmendem Hörverlust nahm also die Anzahl von Fällen mit herabgesetzter kalorischer Reaktion zu.

138 Fälle wurden der Sprachaudiometrie unterzogen und die maximale Diskrimination wurde berechnet. Ein Zusammenhang zwischen Diskriminationsverlust und Herabsetzung der kalorischen Reaktion konnte nicht nachgewiesen werden. Bei 53 Patienten wurde die Höhe der Spitzen in den Békésy Diagrammen mit der kalorischen Reaktion verglichen. Ein Zusammenhang konnte nicht nachgewiesen werden. Geringere Höhe der Spitzen konnte sowohl bei Patienten mit normaler als auch bei Patienten mit fortgeschrittener Abnahme der kalorischen Reaktion auftreten. Der Zusammenhang zwischen den Abständen der Schwelle für kontinuierlichen und gepulsten Ton bei Békésy Audiometrie einerseits und der kalorischen Reaktion andererseits wurde bei 40 Patienten untersucht. Ein deutlicher Zusammenhang konnte nachgewiesen werden. Je grösser der Abstand zwischen den zwei Aufnahmen bei Békésy Audiometrie war, desto grösser war meist auch die Herabsetzung der kalorischen Reaktion.

## REFERENCES

- ALTMAN, F. and KORNFIELD, M. 1963 Histologic studies of Menière disease *Ann. Otol.* 73 813.
- ASCHAN, G., BENNETT, M. and SÄHLIN, J. 1956 Nystagmography—recording of nystagmus in clinical neuro-otological examinations. *Acta Otolaryng.* (Stockh.) 35, 411.
- BETTS, G. 1947 A new diometer. *Acta Otolaryng.* (Stockh.) 35 411.
- CANTERSON, T. E. and HARRIS, R. M. 1953 A comparison between hearing for pure tones and for speech. *J. Laryng.* 67 223.
- DELL, M. R., HALLPIKE, C. S. and HODGE, J. D. 1948 Observations upon the loudness recruitment phenomenon with especial reference to the differential diagnosis of disorders of the internal ear and VIIIth nerve. *Proc. Roy. Soc. Med.* 41 816.
- EST, L. O. and WILLIAMS, H. L. 1951 Recruitment of loudness in the differential diagnosis of end-organ and nerve fibre diseases. *Laryngoscope* 61 100.
- EVANS, A. and SÄHLIN, J. 1967 Hearing in Menière's disease. A study of pure tone diagrams in 334 patients. *Acta Otolaryng.* (Stockh.) 65 543.
- FOWLER, E. P. 1934 A method for the early detection of otosclerosis. Study of sound with bone threshold. *Arch. Otolaryng.* (Chic.) 23 731.
- GOODMAN, A. C. 1945 New observations on changes in hearing in the temporal course of Menière's disease. *Ann. Otol.* 73 991.
- HARRIS, P. and TORVA, I. M. 1962. Clinical applications of hearing tests. *Arch. Otolaryng.* (Chic.) 76, 33.
- HENNINGSON, V. O. 1956 Speed of slow component and duration of caloric nystagmus. *Acta Otolaryng.* (Stockh.) Suppl. 123.
- HODGE, J. D., PAL, A. T. and GOODMAN, A. C. 1954 Difference lines and recruitment. *Arch. Otolaryng.* (Chic.) 60 823.
- HODGE, J. D., and REYNOLDS, J. A. 1952 Recruitment and speech discrimination loss. *Laryngoscope* 62 821.
- JACOB, J. 1960 Audiological manifestations of lesion in the eighth nerve system. *Laryngoscope* 70 417.
- 1960 Békésy audiometry analysis of auditory disorders. *J. Speech Hearing Res.* 3, 273.
- 1962 Hearing tests in otologic diagnosis. *Ashe*, 4 120.
- JONSSON, L. B. W. and PHILIPSSON, A. J. 1964 Electronystagmography. *Acta Otolaryng.* (Stockh.) Suppl. 129.
- HARRINGTON, H. K. 1961 Histopathology of Menière disease. *Acta Otolaryng.* (Stockh.) 53, 237.
- LAVENDER, B. A. 1958 Recruitment measured by automatic audiometry. *Arch. Otolaryng.* (Chic.) 6 835.
- LEDF, G. 1954 Speech audiometry. An experiment in otological study with Swedish language material. *Acta Otolaryng.* (Stockh.) 3, ppl. 154.
- LEVINTHAL, J. H. 1960 Hydrops of the labyrinth. *Arch. Otolaryng.* (Chic.) 71 800.
- 1966 Ménière's disease. Pathology and manifestations. The vestibular system and its disorders. *Trans. of the 1st Symposium. Grad. School Medicine Univ. Pennsylvania, University of Pennsylvania Press, Philadelphia.*
- LIVENSON, T. 1952 Diagnostic problem concerning acoustic phenomena. A study of 340 certified cases, and the Békésy audiogram in the differential diagnosis. *Acta Otolaryng.* (Stockh.) 3, ppl. 99.
- OWEN, O. and FLOTTOR, G. 1957 Menière's disease. Some pathological and clinical observations. *Acta Otolaryng.* (Stockh.) 47 202.
- OWEN, E. 1964 Békésy tracings and the site of lesion. *J. Speech Hearing Dis.* 29 458.
- PAL, A. T. 1956 Recruitment test at low sensation levels. *Laryngoscope* 66 1619.



- PASSAN, E. R. G. 1953: Surgery of the sympathetic for Ménière's disease tinnitus, and nerve deafness. *Arch Otolaryng* (Chic.) 57: 257
- SCHUKMECHT, H. F. 1963: Ménière's disease. A correlation of symptomatology and pathology. *Laryngoscope*, 73: 651
- STAHL, J. 1956: Electronystagmography in the caloric test. *Acta Soc. Med. Upsal.*, 61: 307
- 1958: Electro-nystagmography in the caloric and rotatory tests. A clinical study. *Acta Otolaryng* (Stockh.) Suppl. 137
- 1968: Electronystagmographic results in Ménière's disease. *Otolaryng Clin. North America* Oct. 1968, 509. W. B. Saunders Company Philadelphia
- STAHL, J. and BERGMAN, B. 1967: The caloric reaction in Ménière's disease. An electronystagmographical study in 300 patients. *Laryngoscope* 77: 1629
- STAHL, J. and SAHL, H. 1964: Electronystagmography in Ménière's disease before, during and after ultrasonic irradiation. *Acta Otolaryng* (Stockh.) Suppl. 192: 154

Dept of Otolaryngology  
University Hospital  
Uppsala, Sweden

Received April 10 1968

# HEARING REHABILITATION BY MEANS OF TRANSDERMAL ELECTROTHERAPY IN HUMAN HEARING LOSS OF SENSORINEURAL ORIGIN

## *Preliminary Report*

H. K. PUHARICH and J. L. LAWRENCE

*From the Department of Surgery, New York University Medical Center and  
Intelectron Corporation, New York, N.Y. U.S.A.*

An amplitude modulated radio frequency energy method (the TD system) is described which was applied to the problem of the rehabilitation of sensorineural hearing loss. The TD system was tested on a group of 40 patients with moderate to severe sensorineural hearing loss using a double blind experimental design. The treated group showed a statistically significant improvement in PB word discrimination scores when compared to the control group. Evidence is presented to show that speech discrimination hearing is possible by means of TD electrostimulation in a totally deaf patient.

Over 200 patients with moderate to severe sensorineural hearing loss have been studied during the course of the development of techniques for hearing rehabilitation by means of electrostimulation. The majority of patients have experienced various degrees of improvement in hearing with electrostimulation superior to that found with other conventional means (Puharich & Lawrence, 1964). No undesirable side effects have been found to date as a result of electrostimulation. Clinical experience with electrotherapy in patients with sensorineural pathology by Puharich & Lawrence (1964), Manfredi & Bombelli (1963) and Rottenbard (1964) indicates this to be a promising new technique with potentialities for hearing rehabilitation.

The signal generator is a power amplifier of an amplitude modulated double side band low frequency carrier signal. The output of the generator is coupled to the skin of the patient through an LC series resonant network, and each line terminates on the skin with a plate electrode. Electrodes can be of bare metal, or metal covered with a dielectric material, or a combination of each type.

The electrodes of the transdermal (TD) system are placed across the head of the patient. The carrier signal is brought to peak resonance and is modulated with an audio frequency band that sweeps from 100 Hz to 20 KHz and back again with a four-minute cycling rate. Each discrete audio frequency is cycled every one second from 0-100 per cent modulation.

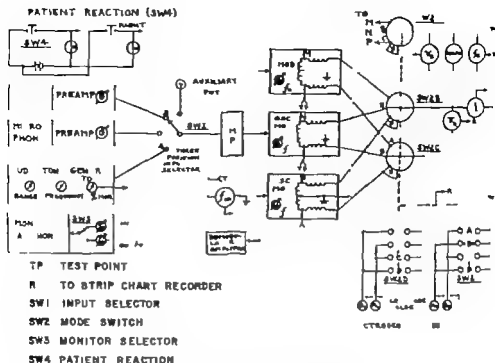


FIG. 1 Clinical transdermal hearing system diagram

and back to 0 per cent with a triangular AM envelope. This energy is applied across the head for a period of about one hour each day for variable periods of time. See Fig. 1 for circuit block diagram.

### EXPERIMENTAL DESIGN AND METHOD

The null hypothesis was "Efficacy of TD treatment is not due to electrotherapeutic effects." Alternate hypothesis "Efficacy of TD treatment is not due to experimental error on the part of the investigators" and/or

Efficacy of TD treatment is not due to experimental error on the part of the patient. This hypothesis was tested by exposing patients to a double-blind TD therapeutic regimen in which pairs of patients would be on placebo or TD treatment for one week.

Patients were selected and evaluated by three independent Board Certified Otolaryngologists, and referred to the experimental TD program at Intellectron. Three audiologists, all Certified for Clinical Competence carried out the audiometric measurements for the three otologists. Each otologist audiologist pair worked independently of each other. The Intellectron staff audiologist, Diana Meyer Certified for Clinical Competence replicated audiometric tests on all of the patients referred by each otologist. Thus, there was obtained an independent set of data by one audiologist on all the patients measured by the three other audiologists. The authors managed the TD treatment program. The distribution of sensorineural hearing loss (as SRT) before the experiment (control) in the 28 patient sample is shown in Fig. 2.

Two TD treatment apparatuses were used as follows:

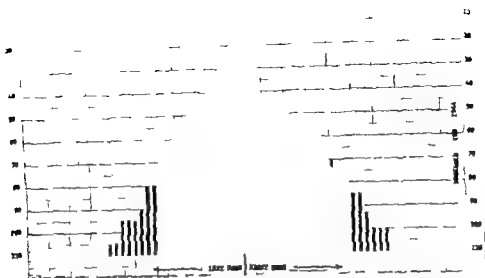


FIG. 2. SRT data (control) taken before manipulation experiment. Histogram shows distribution of SRT levels in 28 patients. The histogram has normal distribution curve for bilateral moderate to severe sensorineural hearing loss. Tall end data below abscissa indicates no response (n.r.) of patient at 110 dB.

1. Two patients were placed on each clinical TD unit at one time. One patient received TD therapy and the other one received an acoustic signal through the headset as a placebo treatment. Both patients wore identical headsets.

2. Setting of the crypto unit in the clinical TD unit was made by a disinterested party and this code was sealed for the first week of treatment.

During the week prior to start of treatment, each patient had two complete audiometry tests. The treatment cycle as outlined in Table 1 was given over the period of four and one half days. One half day was allowed for the patient to visit his otologist for audiometry tests.

At the end of the first week, the seal on the TD crypto unit was broken and it was determined which patient had been on treatment. During the second week, the patient who had been on placebo was TD treated, and the treated patient was kept on TD treatment for a second week. The following week was used to conduct comprehensive audiometric tests for comparison with the initial tests.

No patient was informed before, during, or after the experiment that this was a double-blind experiment. In the treatment program outlined in Table 1 it is to be noted that the control of electrode modes and head position of the electrodes was automatically carried out by a tape program. See Fig. 1 circuit. TD treatment and placebo treatment was identical with respect to audio bandwidth, audio frequency shifts, and audio volume rise and fall

TABLE 1 *Clinical TD treatment program for only one day*

Step	(min) Time	Electrodes	Head position S.M		Head position, tragus		Cyclical AF mod. (Hz)	Series Inductance (mH)
			Right	Left	Right	Left		
1	0-10	Case I A	x ← → x				2000-20	70
2	10-20	Rest						
3	20-30	Case I A	x ← → x				8000-2000	2
4	30-40	Rest						
5	40-50	Case III	Bare ← → Coated				200-8000	30
6	50-60	Rest						
7	60-70	Case III	Bare ← → Coated				200-8000	30
8	70-80	Rest						
9	80-90	Case III	Bare			Coated	200-2000	30
10	90-100	Rest						
11	100-110	Case III	Bare			→ Coated	2000-10,000	30
12	110-120	Rest						
13	120-130	Case III	Bare ← → Coated				200-2000	30
14	130-140	Rest						
15	140-150	Case III	Bare ← → Coated				2000-10,000	30
16	150-160	Rest						
17	160-170	Case II			x ← → x		20-200	30
18	170-180	Rest						
19	180-190	Case II			x ← → x		200-10,000	30
20	190-200	Rest						

since these were taped in advance and, therefore constant for every patient. No speech education or therapy was included in the treatment program. Table 2 shows the total time of active treatment each patient had on the TD machine during this program.

### *Audiometric Tests*

#### *A Pure tones*

Air Conduction 250 Hz, 500 Hz, 1 KHz, 2 KHz, 4 KHz, and 8 KHz.

Bone Conduction 250 Hz, 500 Hz, 1 KHz, 2 KHz, and 4 KHz.

The three outside audiologists used manual techniques testing each ear separately employing white noise masking where applicable descending modified Hughes & Westlake technique. The Intellectron audiologist used an automatic audiometer which uses a modified bracketing technique both ascending and descending each ear tested separately. Critical band masking applied when necessary.

#### *B Speech audiometry*

1 Speech reception threshold—descending technique (in 5 dB steps) testing each ear separately threshold recorded at 50 per cent correct at minimal dB level.

TABLE 2 Patient data.

Female			Male		
Code no.	Age	TD Rx time, hrs	Code no.	Age	TD Rx time hrs
21	20	6.5	24	77	6.5
12	65	6.5	37	61	6.5
17	58	6.5	31	82	6.5
19	19	6.5	30	74	6.5
20	55	13	25	33	13
29	16	13	10	51	13
25	18	13	7	37	13
6	57	13	13	84	13
15	30	13	28	55	13
11	67	13	26	58	13
9	59	13	18	57	13
5	21	20	1	67	27
2	72	26	4	84	26
14	40	26	8	75	0
No. of females: 14			No. of males: 14		

2. Speech detection threshold—descending technique (in 5 dB steps) testing each ear separately threshold recorded at minimal level at which speech was discernable though not intelligible

3. Speech tolerance threshold—ascending technique (in 5 dB steps) testing each ear separately to level at which speech becomes uncomfortable

4. Speech discrimination scores

(a) Discrimination tested at 40 dB above speech reception threshold level, each ear separately. Per cent score recorded based on number per 25 words correct. Masking employed when necessary in contralateral ear

(b) Discrimination at PB maximum determined by increasing or decreasing input in 10 dB steps to level at which patient achieves optimum discrimination per cent out of 25 words. Each ear tested separately masking when applicable

(c) Discrimination at 45 dB dial reading when possible. 25 words presented to each ear and per cent correct recorded

We were interested in those systematic differences that might exist between Intelectron audiometric procedures and those of the participating audiologists in the general sense. For this reason, a set of averages were computed for each pre-treatment test administered by our staff audiologist, and a corresponding set across all three participating audiologists. We focused on the before treatment period since we were interested solely in audiometric, and not treatment, effects. The null hypothesis here is that regardless of the test being administered, variations in the measuring pro-

TABLE 3 (1 and 3) Audiologists matched *t* tests—PB Pct

4 = Intelectron data, A = other's data.

	Control week 0	Control, week 0-1	Control, weeks 1-2
Mean	-4.993	0	4.8 3
S.D.	14.675	11.730	14.176
N	15	16	16
Difference	-4.993	0	4.875
S.E.	3.789	2.933	4.282
D.F.	14	15	15
<i>t</i> -test	-1.302	0	1.139
<i>p</i> =	0.114	1.000	0.2731
	4 - A	A - A	4 - A

There is a significant difference between the data of Intelectron audiologist on the patient sample, and the data of the other three audiologists on the same patient sample.

cedures are randomly distributed, resulting in no significant differences between either set of average scores.

The *t* statistic was used to test for differences between uncorrelated means under the *t* distribution. We accepted the alternate hypothesis, that a difference between staff and participant average scores as large as measured was due to real effects, when the probability of our being wrong in our acceptance was five in a hundred. This level of significance was our criterion for all statistical tests carried out in this analysis.

We did not assume homogeneity of variance between our two sets of data (i.e., that the distribution of individual scores within an average was similar in shape across a set of means) and used the Welch (1947) approximation for heterogeneous variances in all tests of significance between audiologists.

In the ideal experimental design, a complete set of data is collected from each randomly selected subject. Because of the pilot nature of this study, it was not possible to meet this criterion. One reason for missing data was in the limits of the audiometer. With a power range of 0-110 dB, we were limited to using data from those subjects who responded within these limits. Where subjects did not respond, they were scored no response scores.

The final sample size listed with each *t* test computation accounts for those subjects with complete sets of data for that particular test. The computer program which produced our statistics (the data text system)<sup>1</sup> automatically eliminates a subject from the test if any part of his needed responses are incomplete. This does not preclude his contribution to tests of remaining scores in the series.

Computer used IBM 7094 II. Computer program used. The data text system. (1) Computation of *t* test between independent groups, (2) computation of *t* test between matched means.

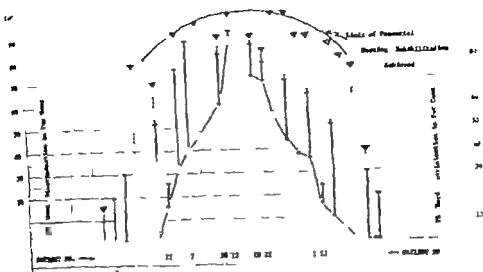


FIG. 3. Symbolic PB discrimination in - 11th acoustic stimulation (post-treatment);  $\nabla$  PB discrimination in - 11th acoustic stimulation (post-treatment); PB discrimination in - 11th acoustic stimulation (pre-treatment).

In planning the experiment we took advantage of the reduction in error variation inherent in employing repeated measures. In this design, the required number of groups are created on the basis of equivalency and random selection. Then after each treatment session, the same group is repeatedly measured for the effect in question. In order to determine if changes have occurred, the *t* test for correlated means is used. It differs from the unrelated version described earlier in that error variation due to non-treatment effect are reduced, thereby increasing the size of the *t* value. Non-treatment effects would be those related to subject idiosyncracies such as test fatigue, nervousness, or other responses which exist across all test situations.

### RESULTS

When we compared audiometric data done by the Intelectron audiologist vs. outside audiologists on the same patient sample with matched *t*-tests, we found that the two sets of data are homogenous for the 15 variables of interest. However, when we look for deviation from week 0 as a result of experimental manipulation during weeks 0-1, weeks 1-2, and weeks 0-2, we find a consistent deviation in only one of the 15 variables, namely PB scores given in percentages. Therefore we look at the audiologists' PB matched *t*-tests in Table 3 for the three time periods in question, weeks 0-1 and weeks 1-2. We find that the two sets of data are homogenous, and, therefore we can analyze either set of data with confidence.

When we look at the outside audiologists' data in Table 4 we see that



TABLE 4

## 1 Treated group

## PB % (other's data)

	TD treated, week 0-1	TD treated, weeks 1-2
Mean	-7.077	0.515
S.D.	9.937	10.511
N	13	11
Difference	-7.077	0.545
S.E.	2.770	3.169
D.F.	12	10
t-test	-2.555	0.172
p =	0.012	0.5 (approx.)

## 2 Placebo group

## PB % (other's data)

	Placebo, week 0-1	TD treated, weeks 1-2
Mean	-2.800	-2.429
S.D.	6.877	8.121
N	10	14
Difference	-2.800	-2.429
S.E.	2.175	2.171
D.F.	9	13
t-test	-1.288	-1.119
p =	0.115	0.161

the treated group for week 0-1 shows significance at the 1 per cent level, and the placebo group for week 0-1 is not significant (at the 11 per cent level). However this result is based on a small  $n$ . In order to further test this result we found an equivalent small  $n$  from the Intelectron audiologists data by using either left ears, or right ears as data source. Table 5 shows such right ear data for week 0-1. It is to be observed that the outside audiologists data and the Intelectron audiologists data are equivalent. Thus, both sets of data show a real treatment effect at work with TD therapy and none in the placebo group.

The power of this argument is amplified in Table 6 where a larger  $n$  is obtained by using only the Intelectron audiologists data. Using Welch's solution (1947) it is found that the critical ratio of difference between the treated group (15.33 PB%) and the placebo group (4.81 PB%) is significant at less than the 1 per cent level. It is concluded that this statistical analysis reveals a real treatment effect for only one of the 15 audiometric variables in the course of a two-week period of experimental manipulation. However it turns out that with respect to the end goal of any hearing rehabilitation therapy, the one variable of utmost importance is that of speech

TABLE 5

*Right ear only — PB Pct. (Intelectron data)*

Treated group

	Week 0-1	Weeks 1-2
Mean	-12.800	-8.000
S.D.	12.621	8.000
N	10	8
Difference	-12.800	-8.000
S.E.	3.991	2.828
D.F.	9	7
t-test	-3.207	-2.828
p =	0.011	0.025

*Right ear only — PB Pct. (Intelectron data)*

Placebo group

	Placebo week, week 0-1	Treatment week, weeks 1-2
Mean	-4.757	-7.000
S.D.	9.069	9.440
N	11	10
Difference	-4.757	-7.000
S.E.	2.741	2.965
D.F.	10	9
t-test	-1.725	-2.345
p =	0.115	0.044

discrimination enhancement. This was the variable that showed a significant enhancement with TD therapy as measured by the PB test.

It was considered desirable to prolong the course of TD therapy in order to see if any of the other variables, in addition to PB%, showed any significant deviation. This question is examined in the next section on a new sample of 12 patients. Since placebo therapy had not shown any significant deviations in the 28 patient sample, the double-blind design was not used in the next study.

#### LONG TERM TD THERAPY

##### Summary of findings

(a) Twelve cases ranging in age from 18 to 83 years with various degrees of sensorineural hearing loss ranging from -40 dB to -110 dB average for pure tones were studied to evaluate potential efficacy and hazard of TD electrostimulation.

(b) TD electrostimulation was given to these patients on the basis of one hour per day TD therapy for 12 to 72 treatments over periods ranging from one day to six months.

(c) Pure tone audiograms were done before TD therapy was initiated, at suitable intervals during therapy and after therapy had ceased. Not a

TABLE 6

## 1 Treated group

## PB % (Intelectron data)

	TD treated, week 0-1	TD treated weeks 1-2
Mean	-15.333	-6.429
S.D.	13.958	1.507
N	18	14
Difference	-15.333	-6.429
S.E.	2.290	3.343
D.F.	17	13
t-test	-4.661	-1.923
p =	0.0001	0.04

## 2 Placebo group

## PB % (Intelectron data)

	Placebo week 0-1	TD treated, weeks 1-2
Mean	-4.818	-6.600
S.D.	.823	10.918
N	23	20
Difference	-4.818	-6.600
S.E.	1.608	2.441
D.F.	21	19
t-test	-2.889	-2.03
p =	0.005	0.004

C.R. of difference for 0-1 vs. 1-2 for group 1 vs. group 2 (Welch Sol)

$$t_{obs} = 3.00 \quad p = < .01 \quad [t_{.05} (30) = 1.70 \quad f = 34.1]$$

single patient showed any loss at any frequency with respect to residual acoustic hearing function as a result of prolonged electrostimulation. No undesirable side effects of electrostimulation were observed or reported.

(d) A criterion of  $\pm 6$  dB was established as a variation for significant improvement or significant loss for pure tone thresholds. The audiogram spectrum was divided into three categories, i.e. low frequency range (12-750 Hz), middle frequency range (1-5 kHz), high frequency range (6-8 kHz). This criterion was applied in each of the three categories to assess improvement or loss in pure tone thresholds following TD therapy. Table 1 shows the results in tabular form. It was concluded that (1) no patient suffered a significant loss in threshold level in any of the three categories, (2) that the greatest lowering of threshold occurred in the low frequency category and (3) that the middle frequency range is the least affected by TD electrostimulation.

(e) For speech audiometry a criterion of  $\pm 10$  per cent was established as the 1 per cent level of significance. It is found that eight of the ears fall

TABLE 7 Pure tone changes

+ 6 dB, or better improvement; 0 6 dB, or less, change; - 6 dB, or more loss. AD = Right Ear AS = left Ear

Case	LL (125 Hz-750 Hz)		MF (1 kHz-8 kHz)		HF (8 kHz-8 kHz)	
	AD	AS	AD	AS	AD	AS
1	+	+	+	0	+	+
2	+	+	+	0	+	+
3	+	0	0	+	0	0
4	0	0	0	0	0	0
5	+	+	+	+	+	0
6	+	0	0	0	0	0
7	+	+	0	0	0	+
8	+	+	0	0	+	+
9	+	0	+	0	+	0
10		+	0	0	0	0
11	0	0	0	0	0	+
12	0	0	0	0	0	0
Totals for twelve patients						
+	9	6	4	2	5	5
0	3	8	8	10	7	7
-	none	none	none	none	none	none
Totals for twenty-four ears						
	15		6		10	
0	9		18		14	
-	none		none		none	

below the 1 per cent level and 16 of the ears exceed the 1 per cent level of significance. It was concluded that 66 per cent of the ears show significant improvement for acoustic speech discrimination.

(f) When we assess acoustic speech discrimination scores before therapy a TD electrostimulation reinforced by an acoustic source after therapy 11 of the ears fall below the 1 per cent level of significance, and 19 of the ears exceed the 1 per cent level. This data is summarized in Fig. 3.

(g) It was found that PB speech audiometry tests could be done at significantly lower levels > 6 dB during the post therapy period as compared to the pre-therapy period.

#### CASE REPORT

R. J. is a 26-year-old white male. Scarlet fever, mumps, and meningitis during the age from two to three years left him totally deaf. R. J.'s eyesight was seriously affected. He was left with minimal residual vision until the age of 20 when he became totally blind.

It is well documented during all the years since R. J.'s meningitis that

TABLE 8 *Protocol of defective hearing cases currently under clinical TD therapy*

PB tests are always given in blocks of 50 taped words from standard lists, except where noted by an asterisk. An asterisk refers to blocks of 10 PB words which the patient learns, and the test is given by randomizing the word order.

Case no	Diagnosis	Age	No. of R	PB a.o. before R		PB speech discrimination post R		PB speech discrimination post R		
				AD %	AS, %	Acoustic AD %	Acoustic AS, %	Transdermal + acoustic AD %	AS, %	Bilateral
1 (S I R.)	AD SN loss. AS deaf (Menière's)	70	72	36	0	64	42	90	40	96
2 (R B)	Congenital SN loss (moderate)	18	12	54	46	96	88	100	96	100
3 (R Mc.)	Infectious SN loss (severe)	38	20	0	0	42	38	76	78	84
4 (P A)	SN loss sec ondary to otosclerosis	27	30	44	32	70	76	100	92	100
5 (S. L.)	AD presby AS deaf (30 yrs dur)	72	24	10	0	50	10	86	16	83
6 (J v H)	SN loss sec. Neomycin. Ototoxicity	48	26	6	0	70	52	80	0	86
7 (G N)	SN loss. Presbycusis. (mod rate)	83	36	38	40	82	88	90	88	92
8 (F S.)	SN loss. Bilateral (total)	58	25	PB = 0 0 Spondi =	0 0 0	30 18	30* 12	40 20	40* 20	40* 20
9 (M P)	SN loss. Bilateral (total)	52	49	0 0 Spondi =	0* 0 0	20 0	20 0	20 16	20* 12	20* 12
10 (D T)	SN loss. Presbycusis. (mild)	77	49	72	60	86	82	90	90	90
11 (J W)	Unsymmetric. bilateral SN loss	56	22	70	16	78	58	84	30	86
12 (W F)	Unsymmetric bilateral (SN loss)	49	14	16	88	24	92	20	100	100

he has been totally anacoustic for pure tones in the frequency range from 125 Hz to 8 kHz at 110 dB pressure levels. His sensitivity to skin vibration was also tested and it was noted that he could sense vibrations up to 1 / kHz.

The use of two bare electrodes was found to be the best technique to deliver the sensation of sound to R. J. when they were placed bilaterally behind the ear  $\frac{1}{2}$  to  $\frac{3}{4}$  inch below the mastoid prominence. The technique used 2 mH chokes and a carrier ranging from 17 kHz to 23 kHz. The current across the head was 2 mA at 2 volts with a total power of 4 mW or less than 1 mW/cm<sup>2</sup>.

An initial test was made of the frequency range of TD response before transdermal therapy. It was found that his response was from approximately 90 Hz to 2.6 KHz. From this time on, successive visits found his range of TD response increased from 70 Hz to 8 kHz. This range was reached in no more than six or seven office visits each lasting no longer than one and one half hours.

With the same bare electrode technique we began to speak words into a microphone and at the same time R. J. was made aware of each word by placing his finger across the speaker's lip, chin, and throat. Initially we started with a group of five or six monosyllable words such as cat, dog, etc. R. J. could learn such a group of words within ten minutes, and when given to him at random, he could score with 70 per cent to 80 per cent accuracy.

This led to more complicated groups such as fan, gun, run, sun, etc. He could repeat these with equal accuracy. No attempt was made to teach R. J. the English language. Words led to sentence acquisition which he could learn as readily as single words and repeat with similar accuracy ( $\approx 80$  per cent).

Since R. J. has been with us for only five months, it is difficult to say at this time what the extent of his language learning capability can be via transdermal hearing. Much work remains to be done. At the end of the five-month period, R. J. is still anacoustic to 110 dB pure tone levels from the acoustic audiometer.

### CONCLUSIONS

A novel electrotherapeutic technique, the Transdermal system (TD), was developed. It was applied to the problem of hearing rehabilitation in hearing loss of sensorineural origin in a group of 40 patients.

Twenty-eight patients with sensorineural hearing loss were subjected to a one week double blind course of TD electrotherapy. Patients receiving placebo therapy showed an average five per cent increment in PB word discrimination scores, and this is ascribed to test, retest learning (Group 2). Patients receiving active TD electrotherapy showed an average 17 per cent increment in PB word discrimination scores (Group 1). The critical tail difference between Group 1 and Group 2 is significant at  $p = < 0.01$ .

Twelve other patients with sensorineural hearing loss were then subjected

TABLE 2 *Protocol of defective hearing cases currently under clinical TD therapy*

PB tests are always given in blocks of 50 taped words from standard lists, except here noted by an asterisk. An asterisk refers to blocks of 10 PB words which the patient learns, and the test is given by randomizing the word order

Case no	Diagnosis	Age	No of R	PB speech discrimination post R <sub>1</sub>						
				PB s D before R		Acoustic		Transdermal+acoustic		
				AD %	AS, %	AD %	AS, %	AD	AS, %	Transdermal
1 (S. I R)	AD SN loss. AS deaf (Menière's)	70	72	36	0	64	42	90	40	96
2 (R. B)	Congenital SN loss (moderate)	18	12	54	46	96	88	100	96	100
3 (R Mc.)	Infectious SN loss (severe)	38	20	0	0	42	38	76	78	84
4 (P A)	SN loss sec ondary to otosclerosis	27	30	44	32	70	6	100	92	100
5 (S. L.)	AD presby AS deaf (30 yrs dur)	72	24	10	0	50	10	86	16	84
6 (J v H)	SN loss sec. Neomycin. Otototoxicity	48	26	0	0	70	52	80	70	88
7 (G N)	SN loss. Presbycusis. (moderate)	83	36	36	40	82	66	90	88	90
8 (P S.)	SN loss. Bilateral (total)	58	25	PB = 0 0	0	30	30	40	40	46*
				Spondi =		18	12	20	20	28
9 (M P)	SN loss. Bilateral (total)	52	49	0*	0	20	20	20	20	20*
				Spondi =		0	0	16	12	16
10 (D T)	SN loss. Presbycusis. (mild)	77	49	72	60	86	82	90	90	90
11 (J W)	Unsymm tric bilateral SN loss	56	22	70	16	8	26	84	30	86
12 (W F)	Unsymm tric bilateral (SN loss)	49	14	16	88	24	92	20	100	100

## ZUSAMMENFASSUNG

Die amplitudenmodulierte Radiofrequenz-Energie-Methode (das sogenannte TDS-System) ist geschildert die beim Problem der Rehabilitation (bzw. Wieder-Einstandsetzung) des sensorischen Hörverlustes angewandt wurde. Das TDS-System wurde an einer Gruppe von 40 Patienten ausprobiert die in eine Probestudien-Gruppe und eine Kontrollgruppe eingeteilt waren. Diese Patienten hatten mäßigen bis starken Hörverlust der sensorischen Nerven. Die behandelte Gruppe zeigte eine statistisch bedeutende Verbesserung gegenüber der Kontrollgruppe in PB-Word-Unterscheidungs-Ergebnissen. Es wird bewiesen, dass sprachunterscheidendes Hören bei einem vollkommenen Hören Patienten möglich ist mit Anwendung der TD-Elektrostimulation.

## REFERENCES

- JEN, GORD, ROSEY, S., SCHULZ, J. PLESTER, D. and EL-MORIT, A. 1964 Vegetative reactions to auditory stimuli. *J. Amer. Acad. Ophthalmol. Otolaryng.* 1, 445.
- MAFAROT, A. and BOWEN, J. 1963 Radioacoustic treatment of deafness. *Publicazioni dell'Istituto Nazionale di Ottica*, 4, 400, 403.
- PIKARICH, H. K. and LAWRENCE, J. L. 1964 Alternating current energy and stimulation of deaf humans. Paper presented at 35th Annual Convention, Aerospace Medical Association, Miami, Florida.
- 1964 *Electrostimulation Techniques of Hearing* Technical documentary report No. MADC-YDR-44-18, prepared under contract No. AF30(602)-3031, project No. 5334 task No. 533401 Defense Documentation Center AD No. 459834, Cameron Station, Alexandria, Va.
- ROTEVNIK, S. R. 1964 *Electroloop Clinical Data. A manual* p. 72. V. Med. pers., Moscow USSR.
- ROSEY, S., BERGMAN, N., PLESTER, D., EL-MORIT, A. and KATZ, M. H. 1962 Presbycusis study in relatively noise-free population in the Sudan. *Am. J. Otol.* 7, 727.
- ROSEY, S. and OLIVIER, A. 1963 Hearing loss and coronary heart disease. *Arch. Otolaryng.* (Chic.) 82, 228.
- ROSEY, S., PLESTER, D., EL-MORIT, A. and ROSEY, H. 1964 Relation between loss of cardiovascular disease. *Trans. Am. Acad. Ophthalmol. Otolaryng.* Vol. 422.
- TROWELL, T. 1966 *Biological Membranes. Recent Progress* (ed. Werner K. Loewenstein) p. 936. Electrokinetic consideration of mechanoelectrical transduction. *Ann. NY Sci.* 137, pt. 2, July 14, 1966, p. 463.
- WELCH, B. L. 1947 The generation of St. dent's problem when several different population classes are involved. *Brown trike*, 21, 23.

Dept. of Surgery New York  
University Medical Center 60  
First Avenue New York NY  
10016, U.S.A. and Electron  
Corporation, 432 West 45th  
Street, New York NY 10036  
U.S.A.

Received April 27 1968



to a prolonged course of TD electrotherapy. They showed an average 21 per cent increment in PB word discrimination scores.

Ten animals were studied in the following three areas. TD electrostimulation of hearing in two totally deaf dogs showed evidence for hearing neuropathological studies of the brains of two dogs subjected to chronic (320 hours) TD electrostimulation revealed no gross or microscopic abnormalities and cardiovascular physiological studies of acute (eight hours) TD electrostimulation in six dogs showed no abnormal effects. It is concluded that TD electrostimulation is safe for humans within the power and time exposure applied.

A case report is given on the results of TD electrotherapy and TD hearing electrostimulation with a totally deaf patient. Evidence is presented to show that speech discrimination hearing is possible in a totally deaf patient by means of TD electrostimulation.

There are two main mechanisms to consider. The first is to try to explain sensorineural rehabilitation resulting from TD electrotherapy. The second is to explain speech discrimination in the totally deaf human by means of TD electrostimulation.

With respect to the first, human and animal studies have shown that the application of amplitude modulated AC energy (the TD signal) to tissues creates a DC barrier potential of 0.5 volt. The induced barrier potential is negative in sign inside of cells, and positive on the outside. The exception to this rule is that red blood cells, leukocytes and platelets, accumulate a negative charge on the outside surface. This effect prolongs blood clotting time *in vitro* by a factor of three compared to controls that were not charged with the TD signal. Microscopic observation shows that TD charged red blood cells are repelled from each other by negative charge repulsive forces. Rosen *et al* (1962, 1964, 1965) have stressed the importance of circulatory efficiency in the cochlear system with respect to good hearing and to hearing loss. In personal communications with Dr. Rosen, a plan was formulated to evaluate the effects of TD electrotherapy on cochlear circulatory factors. This may be one of the more important effects of clinical TD therapy which leads to improved speech discrimination hearing.

With respect to speech discrimination by means of TD electrostimulation in the deaf, the problem is very complex. It has been established that the induced DC barrier potential is modulated by the audio envelope of the amplitude modulated carrier signal. How this effect can substitute for the cochlear transduction and encoding processes is not yet clear. Various models for such a cochlear function analog are now being explored by computer simulation. One of the more promising models being investigated is the artificial pressure-receptor analog of Teorell (1966).

such examples as the electromyographic studies described by Fanborg-Andersen (1965) on humans and the cortical study by Hast & Milojevic (1966) on the primate most of our knowledge of the physiological mechanisms of the intrinsic muscles of the larynx has been derived from research on the domestic dog and cat (Fessard & Vallboen, 1957 Floyd *et al* 1957 Dunker & Schlosshauer 1958 Iashiki, 1959 Mårtensson, 1963, 1964 Mårtensson & Skoglund, 1964 Kirchner & Wyke 1965 Hast, 1961 1964 1965 a, 1966 ■ 1967 a 196 b) This fact does not necessarily lessen the value of past experimental findings. But this researcher believed that it would be most fruitful to expand our explorations into the realm of man's closest homologue. We are fortunate in having as our base the excellent comparative anatomical studies of Sir Victor Negus (1929) and the reader is referred to this text as a source for a detailed description of most animal larynges.

The purposes of this study were (1) To measure the mechanical properties of the thyroarytenoid and cricothyroid muscles in three primates, the Hyman gibbon (*hylobates hainanus*) rhesus Macaque (*macaca mulatta*) and squirrel monkey (*saimiri sciureus*) and (2) to compare findings from primate research to previously published data derived from experiments on dogs and cats.

#### MATERIAL AND METHOD

Because of the great expense and difficulty in acquisition, only two gibbons (an anthropoid ape) were used in this study the male weighed 7 kg and the female 4 kg Experiments were also performed on 5 chest squirrel monkeys of both sexes their average body weight was 3.6 kg (range of 3-4.3 kg) and 670 g (range of 400-850 g) respectively All animals were anesthetized intraperitoneally with pentobarbital sodium (20 to 25 mg/kg) As a consequence of the size of the laryngeal organ in the rhesus and squirrel monkey (Fig 1) all operative procedures on these animals were performed with the aid of a Zeiss operation microscope and special microsurgical instruments.

Animals were placed in a supine position, tracheostomy was performed to maintain a free airway and the larynx exposed from the hyoid bone to the second or third tracheal ring. The recurrent laryngeal and extrinsic branch of the superior laryngeal nerve was freed unilaterally by use of a very fine dissecting scalpel and dissecting needle.

A ventromedian incision was then made through the larynx from the hyoid to the first tracheal ring. This was followed by two mediolateral incisions of the thyroid cartilage one superior and one inferior to the vocal fold the origin of the thyroarytenoid muscle was finally freed by sectioning the thyroid cartilage perpendicular to and between the mediolateral incisions. A length of silk was tied into the cricoid attachment of the cricothyroid muscle and another into the thyroid attachment of the thyroaryte-

## THE PRIMATE LARYNX

### *A Comparative Physiological Study of Intrinsic Muscles*

M H HAST

*From the Department of Otolaryngology and Maxillofacial Surgery College of  
Medicine University of Iowa Iowa City Iowa U.S.A*

A study was made of the mechanical properties of the thyroarytenoid and cricothyroid muscles in three primates Squirrel monkey rhesus Macaque and Hyman gibbon The thyroarytenoid and cricothyroid muscles were stimulated electrically through their respective motor nerve supply Isometric contractions of muscles were recorded by a force transducer and high-speed inkwriter Measurements were made of the contraction time half relaxation time tetanus, and tetanus twitch tension ratio of each muscle It was found that the thyroarytenoid possessed the properties of a "very fast" muscle (14 msec) and the cricothyroid of the gibbon and rhesus those of a muscle of "average" speed (38 msec) With the exception of the cricothyroid muscle in the squirrel monkey (19 msec) there were no real differences among the three primates studied Comparative values of both laryngeal muscles were similar for the domestic dog and primate but not for the cat whose laryngeal muscles had been found to be physically slower The physiological similarity of the dog and the primate larynx substantiates the dog's value as an experimental analogue for studies in muscle physiology of the larynx

When one considers that the greater part of our knowledge of the science of physiology is derived from observations of laboratory experimentation on animals, it behooves the researcher to continually reinvestigate his principal source of material through a study of comparative anatomy and physiology Aside from the idealism of seeking a basic truth the medical scientist assumes a responsibility when pursuing his discipline the acquisition of knowledge for the betterment of human health Hence the scientist attempts to apply findings of basic research to man He must, however relate experimental data cautiously fully aware that differences between species may limit the application of comparative findings to human function

The above discussion is of singular importance in the study of the neuromuscular mechanisms of the laryngeal organ With the exception of

This study was supported by USPHS Research Grant 05019 from the National Institute of Neurological Disorders and Blindness.



Fig. 2. The stimulating electrode used in this study. Dimension of the electrode-head 2.7 mm. 3 mm.

traction was established, the amplitude of the electrical stimulus was increased to a supramaximal voltage of 1-2 V. The nerves were stimulated with square wave pulses of a duration of 0.1 msec at a frequency of 1-150/sec (Grass S4G stimulator). Trains of stimuli were limited to 2 sec and an interval of 10 sec was maintained between successive tetani to allow for dissipation of post tetanic potentiation of muscle twitches.

## RESULT

The results of all experiments are given in Table 1. Examples of mechanical properties of each muscle are shown in Figs. 3, 4 and 5.

An examination of the data reveals that for gibbon, rhesus, and squirrel monkey the average contraction times for the thyroarytenoid muscle were similar. One variant should be noted, however, the very fast response of 8 msec by one of the squirrel monkeys. There was also no real difference between average values for the mechanical properties of the cricothyroid muscle in the gibbon and rhesus. The notable exception was the faster contraction time of the squirrel monkey. The mean values from this author's past research, published (Haas, 1966, 1967a) and unpublished, on dogs and cats is presented in order that a comparison can be made between these two common laboratory animals and the three primates. An examination of Table 1 revealed that mechanical properties of the dog's laryngeal muscles were quite similar to that of the primates. This is not true for the thyroarytenoid and cricothyroid muscles of the cat.

Not shown in Table 1 was the force exerted by these muscles during an isometric contraction at *in situ* resting length. For the rhesus monkey the average tetanus twitch tension ratio (g wt.) was 51.4 g/7.1 g for the

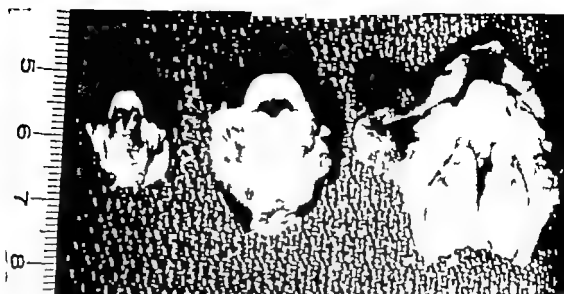


FIG. 1 A dorsal view of the three primate larynges studied. From left to right: Squirrel monkey, rhesus, and gibbon.

noid muscle. To further isolate the thyroarytenoid muscle from the interaction of other intrinsic muscles, the arytenoid cartilage was sutured to the thyroid cartilage. With the gibbon ties were made with 4-0 surgical suture because of the limitations of size in the case of the rhesus and squirrel monkey, ties were made with 6-0 silk armed with a micro-point cutting needle (Ethicon).

The free end of each piece of silk was tied to a force displacement transducer (Grass FT 003 c) attached to a heavy stand. The output signal of the transducer was amplified and recorded by a high speed inkwriter (Offner RS Dynograph). Isometric recordings were made of each muscle separately. For all primates, measurements of the mechanical properties of contraction time, one half relaxation time, tetanus, and tetanus/twitch tension ratio were taken at the *in situ* resting length of each muscle. Since the speed of muscle contraction has a high temperature coefficient ( $Q_{10}=1.53$ ) according to Gordon & Phillips (1953) temperature was maintained at  $37^{\circ}\text{C}$  ( $\pm 0.2$ ) by the heat of an electric lamp and monitored by a thermistor probe connected to the circuit of a calibrated electrical thermometer. The preparation was bathed by a physiological irrigating solution. It should also be noted that the *in situ* resting length was maintained since the progressive lengthening of a muscle would increase the contraction time of the twitch (Buller *et al.*, 1960).

The laryngeal nerves were attached to a very small shielded bipolar platinum electrode and bathed in a pool of warm mineral oil. The electrodes used in this study were designed by the author and constructed by a skilled craftsman at our institution (Fig. 2). After the threshold of muscle con-

The electrode shown in Fig. 2 was made by M. J. H. A. Dysan, Ph.D., Department of Physiology, Medical Laboratory, University of Iowa, Iowa City, Iowa.

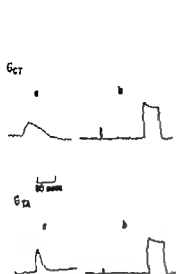


FIG. 2.

FIG. 2. Recordings of mechanical properties of two intrinsic laryngeal muscles of gibbon no. 1:  $G_{CT}$  Cricothyroid muscle;  $G$  thyroarytenoid muscle; a, single twitch response for calculating contraction and half-relaxation times; b, twitch and tetanic responses from which the tetanic twitch tension ratio was computed.

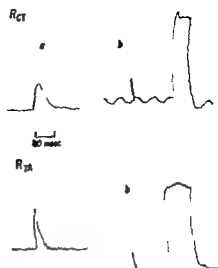


FIG. 4.

FIG. 4. Recordings of mechanical properties of two intrinsic laryngeal muscles of rhesus no. 2:  $R_{CT}$  Cricothyroid muscle;  $R_A$  thyroarytenoid muscle; a, single twitch response for calculating contraction and half-relaxation times; b, twitch and tetanic responses from which the tetanic twitch tension ratio was computed.

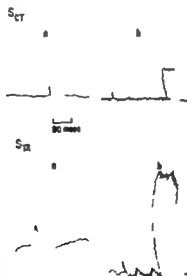


FIG. 5. Recordings of mechanical properties of two intrinsic laryngeal muscles of squirrel monkey no. 4:  $S_{CT}$  Cricothyroid muscle;  $S_A$  thyroarytenoid muscle; a, single twitch response for calculating contraction and half-relaxation times; b, twitch and tetanic responses from which the tetanic twitch tension ratio was computed.

TABLE 1 *Mechanical properties of the cricothyroid and thyroarytenoid muscles of the Hyman gibbon rhesus Macaque and squirrel monkey*

		Cricothyroid				Thyroarytenoid			
Sex	Weight	Contraction time msec <sup>a</sup>	1/2-relaxation time msec	Tetanus (pulses/sec)	Tetanus Twitch tension ratio	CT	1/2-RT	Tet	T T
<i>Hyman gibbon</i>									
1	m	70 kg	40	36	40	3.0 1	16	16	100 62 1
2	f	40 kg	48	36	45	3. 1	16	18	90 55 1
Average		39	36	43	3.4 1	16	17	90	59 1
<i>Rhesus Macaque</i>									
1	m	3.0 kg	28	20	60	5.4 1	12	16	90 1 1
2	m	3.4 kg	44	24	40	4.2 1	14	12	110 53 1
3	f	3.0 kg	38	24	45	4.5 1	14	16	110 61 1
4	f	4.1 kg	40	24	50	3.9 1	16	14	130 102 1
5	m	4.3 kg	32	24	50	4.2 1	14	14	120 70 1
Average		36.4	23.2	49	4.4 1	14	14.4	112	1 1
<i>Squirrel monkey</i>									
1	m	850 g	22	14	63	4.1 1	16	14	90 44 1
2	m	650 g	16	16	80	4.4 1	16	14	90 42 1
3	f	800 g	24	14	70	4.2 1	12	16	120 58 1
4	f	400 g	16	12	90	4.6 1	8	10	150 98 1
5	m	680 g	16	12	110	5.5 1	14	16	130 60 1
Average		18.8	13.6	83	4.6 1	13.2	14	116	60 1
<i>Domestic dog</i>									
(average of 10)		39	32.3	45	4.2 1	14	18	114	62 1
<i>Domestic cat</i>									
(average of 10)		52.8	42.1	37	3.7 1	22	20	73	50 1
± 1 msec									

thyroarytenoid muscle and 22.8 g 5.2 g for the cricothyroid muscle. With the squirrel monkey the average tension was 3.4 g 610 mg for the thyroarytenoid muscle and 2.3 g 512 mg for the cricothyroid muscle. These values for muscle tension serve to illustrate the several problems that can arise when dealing with such small masses of tissue, particularly in experiments with the squirrel monkey.

## DISCUSSION

With the exception of the cricothyroid muscle of the squirrel monkey average values for the mechanical properties of both muscles revealed no real differences among the primates studied. Values for the average contraction times of 19, 30, and 39 msec for the cricothyroid muscle of the

the two levels, each best suited to perform a particular function. By this means adaptations for a particular purpose can take place at one level without sacrificing anatomical requirements for a different but equally important function which can continue to take place at the second level. For instance had a single powerful sphincter band been retained, it would of necessity have been either too gross a structure to produce a pleasant variable voice or if it had developed as a single delicate band capable of producing graded tones over a long range such delicacy of structure would in all probability have rendered it ineffective as a powerful sphincter. It is, incidentally interesting to note that the greater physiological importance of the sphincteric mechanism is hinted at in nature by the fact that in species in which the larynx is designed primarily for only one purpose, the anatomic structure of the organ is better adapted to the sphincter action than to the production of sound.

The very real differences observed in this study between the laryngeal muscles of the cat and those of man's closest homologues, the monkey and ape may open to question the value of the cat as an experimental analogue. On the other hand, the physiological similarity of the dog and the primate larynx substantiates the dog's value for research on the neuromuscular mechanisms of the larynx.

#### ZUSAMMENFASSUNG

Die mechanischen Eigenschaften der Thyreoaryten- und Cricothyroideus-Muskeln wurden in drei Primaten untersucht: *Haplorhina aethiops*, *Macaca mulatta*, *Saimiri sciureus* s. Diagonale Muskeln wurden über ihr jeweiliges Bewegungsnervensystem elektrisch gereizt. Die isometrischen Kontraktionen der Muskeln wurden durch einen Kraft Transducer und einen Hochfrequenz-Tinten-schreiber aufgeschrieben. Gemessen wurden die Kontraktionszeit, die Halb-Relaxationszeit, die Tetanusfrequenz sowie das Tetanus-Zerspannungsverhältnis jedes Muskels. Der M. thyreoarytenoideus erwies sich als ein „sehr rascher“ Muskel (14 msec) und der M. cricothyroideus des Gibbon und Rhesusaffen als ein Muskel von „mittlerer“ Geschwindigkeit (38 msec). Mit Ausnahme des M. cricothyroideus des *Saimiri larus* (10 msec) verliefen die Untersuchungen mit den 3 Primaten hauptsächlich parallel zu früheren Vergleichswerten für die beiden Kehlkopfmuskeln bei Hunden. Die Primaten waren ähnlich, leicht aber bei Katzen deren Muskeln auch bei früheren Versuchen als langsamer erwiesen haben. Die physiologische Ähnlichkeit der Kehlköpfe von Hund und Primat bestätigt den Wert des Hundes als experimentelles Analogon in Untersuchungen der Physiologie der Kehlkopfmuskeln.



squirrel monkey, rhesus, and gibbon respectively do not agree with the published findings of 20 msec by Mårtensson & Skoglund (1964). A problem arises, though, in trying to compare this work with that of Mårtensson & Skoglund (1964) for to use their own words, only certain muscles of the cat and dog were systematically studied [with] occasional readings made on monkey. (The number and species of monkey was not given.)

The squirrel monkey with its very high pitched voice not only possesses a "fast" cricothyroid muscle but a vocal fold muscle capable of achieving an extremely high speed of contraction. Judging from the 8 msec value of squirrel monkey no. 4. In this particular animal the thyroarytenoid muscle was comparable in speed of contraction to the extraocular medial rectus of the cat (Cooper & Eccles, 1930).

One of the difficulties in attempting to correlate the data among the three animals is the limited number of the experiments performed on the gibbon. This researcher believed however that since there has been little published data on the physiological properties of primate laryngeal muscle findings from experiments on this anthropoid ape, no matter how limited, would be of interest to physiologists and laryngologists.

There is another observation which must be discussed and here again the reader is referred to Table 1. Values for the mechanical properties of the primates' intrinsic laryngeal muscles were similar to those of the domestic dog but not the cat. This investigator found the thyroarytenoid (Hast 1967a) and cricothyroid (unpublished) muscles of the cat to be physiologically "slower" than comparable muscles in the dog. Mårtensson & Skoglund obtained reverse results. Values for the contraction times of these laryngeal muscles in the dog (Hast 1966a, 1967) do agree however with the findings of Mårtensson & Skoglund (1964). One cannot account for the large and contrary differences found between this study and the previous work (cricothyroid 30-35 msec, thyroarytenoid 9-13 msec) for values of cat laryngeal muscles, especially since the techniques used by this researcher were quite similar to those of Mårtensson & Skoglund. Again, it is difficult to compare studies, since those of the other authors reported values for only one mechanical property. In contradistinction to the four measurements made on each muscle by this investigator.

Comparative gross and microscopic anatomical studies, by this and other, equally important function which can continue to take place at the physiological findings. For example the laryngeal sphincter of the cat is represented by a single valve. In contrast to the double sphincter (false and true folds) of the dog, monkey, ape, and man. In their monograph Pressman & Kellman (1955) offer some interesting speculations on the significance of this major structural and evolutionary difference.

It is well that in man such a division took place for it provides the advantage of permitting differences in anatomical construction at

the two levels, each best suited to perform a particular function. By this means adaptations for a particular purpose can take place at one level, without sacrificing anatomical requirements for a different but equally important function which can continue to take place at the second level. For instance had a single powerful sphincter band been retained, it would of necessity have been either too gross a structure to produce a pleasant variable voice or if it had developed as a single delicate band capable of producing graded tones over a long range, such delicacy of structure would in all probability have rendered it ineffective as a powerful sphincter. It is, incidentally interesting to note that the greater physiological importance of the sphincteric mechanism is hinted at in nature by the fact that in species in which the larynx is designed primarily for only one purpose the anatomic structure of the organ is better adapted to the sphincter action than to the production of sound.

The very real differences observed in this study between the laryngeal muscles of the cat and those of man's closest homologues, the monkey and ape may open to question the value of the cat as an experimental analogue. On the other hand the physiological similarity of the dog and the primate larynx substantiates the dog's value for research on the neuromuscular mechanisms of the larynx.

#### ZUSAMMENFASSUNG

Die mechanischen Eigenschaften der Thyreoarytenoideus- und Cricothyroideus-Muskeln wurden bei drei Primaten untersucht: *Hyllobates kalmannus*, *Macaca mulatta*, *Salomys sciurus*. Die genannten Muskeln wurden über ihr jeweiliges Bewegungssystem elektrisch gereizt. Die isometrischen Kontraktionen der Muskeln wurden durch isometrische Krafttransducer und einen Hochfrequenz-Tintenschreiber aufgenommen. Gemessen wurden die Kontraktionszeit, die Halblaxationszeit, die Tetanusfrequenz sowie das Tetanus-Zerspannungsverhältnis. Jedes Mal, da der M. thyreoarytenoideus erwiesen sich als ein sehr schneller Muskel (14 msec) und der M. cricothyroideus des Cibbon und Rhesusaffen als ein Muskel von „mittlerer“ Geschwindigkeit (38 msec). Mit Ausnahme des M. cricothyroideus des *Salomys sciurus* (19 msec) verliefen die Untersuchungen mit den 3 Primaten ohne wesentliche Unterschiede. Vergleichswerte für die beiden Kehlkopfmaskeln bei Hu und Primaten waren ähnlich, nicht aber bei Katzen, deren Muskeln sich bei früheren Versuchen als langsamer erwiesen haben. Die physiologische Ähnlichkeit der Kehlkopf von Hu und Primat bestätigt den Wert des Hundes als experimentelles Analogon in Untersuchungen der Physiologie der Kehlkopfmaskeln.

## REFERENCES

- BULLER, A. J., ECCLES, J. C., and ECCLES, R. M. 1960 Differentiation of fast and slow muscles in the cat hind limb. *J Physiol* 150 399.
- COOPER, H. and ECCLES, J. C., 1930 The isometric responses of mammalian muscles. *J Physiol* 69 37.
- DUNKER, E., and SCHLOSSHAUER, B. 1938 Klinisch und experimentelle Studien über Stimmlippenschwingungen. *Arch. Ohr Nas Kehlkopfheilk* 172 363.
- FAABORG-ANDERSEN, H. 1963 *Electromyography of Laryngeal Muscles in Humans: Technique and Results*. Vol. 3, current problems in Phoniatrics and Logopedics, S. Karger, Basel.
- FESSARD, A. and VALLA CHER, B., 1961 Sonde électrophysiologique sur le fonctionnement de l'appareil phonatoire du chien: étude des effets de la stimulation du nerf laryngé supérieur. *Folia Phoniat (Basel)* 9 162.
- FLOYD, W. F., NEGRO, V., and NEIL, E., 1957: Observations on the mechanism of phonation. *Acta Otolaryng (Stockh.)* 48 111.
- GORDON, G. and PHILLIPS, C. G. 1953 Slow and rapid components in flexor muscle. *Quart. J. Exp. Physiol.* 38 35.
- HART, M. H. 1961: Subglottic air pressure and neural stimulation in phonation. *J. Appl. Physiol.* 16 1142.
- 1964: Chronaxie of the vocal fold of the dog. *Ann. Otol.* 73 305.
- 1966a: Mechanical properties of the cricothyroid muscle. *Laryngoscope* 6 53.
- 1966b: Physiological mechanisms of phonation: tension of the vocal fold muscle. *Acta Otolaryng (Stockh.)* 62 309.
- 1967a: Mechanical properties of the vocal fold muscle. *Pract. Otorhinolaryng (Basel)* 29 53.
- 1967b: The respiratory muscle of the larynx. *Ann. Otol.* 6 489.
- HART, M. H. and MILOJEVIC, B. 1966: The response of the vocal folds to electrical stimulation of the inferior frontal cortex of the squirrel monkey. *Acta Otolaryng (Stockh.)* 61 196.
- ISRAEL, V. 1959: Regulation of the pitch and volume of voice. *Otol. Rhinol. Laryng. Clin. Exptl.* 5 1063.
- KELEMAN, H. 1938: Physiology of phonation in primates. *Logos* 1 32.
- 1961: Anatomy of the larynx as a local organ evaluated in respiratory aspects. *Logos* 4 46.
- KIRCHER, J. A. and WELZ, B. D. 1966: Articulatory reflex mechanism of the larynx. *Ann. Otol.* 4 749.
- MARTENSSON, A. 1963: Reflex responses and recurrent discharges evoked by stimulation of laryngeal nerves. *Acta Physiol. Scand.* 57 248.
- 1964: Proprioceptive impulse pattern during contraction of the laryngeal muscles. *Acta Physiol. Scand.* 69 176.
- MARTENSSON, A. and SÖLUND, L. R. 1964: Contractile properties of intrinsic laryngeal muscles. *Acta Physiol. Scand.* 60 318.
- NEGRO, V. E., 1959: *The Mechanism of the Larynx*. H. K. Lewis, London.
- PRESTON, J. J. and KELEMAN, H. 1935: Physiology of the larynx. *Physiol. Rev.* 35 506.
- WELZ, B. D. 1956: The mechanical properties of muscle. *Bull. Med. Bull.* 12 177.

*Dept. of Otolaryngology and  
Maxillofacial Surgery, Lister  
Institute, 11th Floor, 11th City  
11th 5210, U.S.A.*

Received May 27 1968

ACTA OTO LARYNGOLOGICA

Vol 67 Fasc. 2-3

COMPTE RENDU  
DE LA RÉUNION SCIENTIFIQUE DU

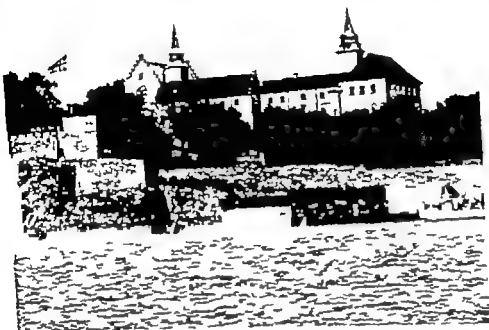
COLLEGIUM  
OTO RHINO LARYNGOLOGICUM

AMICITIAE SACRUM

OSLO LE 19-20 AOÛT 1968

COPYRIGHT BY ACTA OTO LARYNGOLOGICA  
VARVÄGEN 16, 113 23 STOCKHOLM

*Almqvist & Wiksells*  
BOKTRYCKERI AKTIEBOLAG  
UPPSALA 1969



## INTRODUCTION

HANS VOGT

*Oslo, Norway*

Ladies and Gentlemen

The president of this distinguished society has asked me to say a few words at the opening of this congress. It was a pleasure to accept this invitation and to have the opportunity of bringing you the greetings of the University of Oslo, which I have the honour to represent.

Congresses have an important function in the scientific life in our time. The mass of information published every year is so enormous that no man can hope to cope with it singlehanded. The solution to this problem is very often personal contact with colleagues, co-workers, and friends who can share their knowledge so that each of them can pick up just that piece of information he needs. Congresses can serve the purpose of creating contact over and across national boundaries—on the condition however that they succeed in keeping the number of participants within reasonable limits. Con-

gresses with many thousands of participants—6000 mathematicians at the last international congress of mathematicians in Moscow—defeat their own purpose

You have, as far as I know in your society and at your annual meetings stressed quality more than quantity friendship and cooperation more than competition and rivalry That should in itself be a guarantee for the success of the work you will do in the days to come

On behalf of the University of Oslo I bring you best wishes for the congress, and declare the congress open.

# TITRATION OF HORMONE METABOLITES IN CASES OF NASOPHARYNGEAL ANGIOFIBROMA FIVE YEARS AFTER THE OPERATION

S KARATAY S. KATIRCIÖGLÜ and O ERÖZDEN

*Istanbul Turkey*

At the 1963 meeting seven nasopharyngeal angiofibromas were reported from the endocrinological point of view. Four cases out of seven were followed for five years. Recent investigations of their hormone metabolites revealed that in the tumor-free cases the hormone-metabolite titrations had improved. In cases with the tumor still present, findings were the same as at the beginning.

During the Collegium meeting in Athens in 1963 we reported an endocrinologic study of eight cases of nasopharyngeal angiofibroma concerning the relationship between the sex hormones and the pathogenesis of the tumor (Karatay *et al.*, 1963).

On that occasion we tried to demonstrate that there was always an androgen-estrogen imbalance in these cases. Upon the post-operative death of one of those cases we had the opportunity to study histologically the patient's endocrine glands and gonads (which were also described in the abovementioned paper).

The seven cases were kept under control for five years after the operation and they were given additional hormone preparations. Only four of them, however, came to their last control in 1968. As a complementary study to the first paper this short contribution will deal with the follow up of the laboratory findings of these cases, obtained in the last controls.

As we stated before we believe that nasopharyngeal angiofibroma is a male pathology. From 1935 up to 1968 we observed in our clinic 33 cases. The first female case was hospitalized only this year (1968) with a biopsy diagnosis of angiofibroma. After the operation material was once more sent for histological study and this time a retinoblastoma was diagnosed. Hormone metabolites of this case which were studied before the operation, were also found normal.

## LABORATORY FINDINGS

According to the findings of our follow-up cases, we can state that following surgical removal of the tumor and post-operative treatment with hormone preparations (Testosterone, 25 mg twice a week for a period of 3-4 months) the androgen-estrogen balance returned to normal after a time in the tumor-free cases.



TABLE 1

Case		1 Co mg	17 OH mg	Folliculin-gamma
1	8 <sup>a</sup>	2.10-1.88	1.37-1.64	1.8-2.6
2.	5	4.90-8.5	3.96-7.5	27-20
3	4	5.2-9.7	3.43-8.5	35-15
4	2	5.13-0.80	2.8-5.45	30-70
Normals		12-18	5-9	0- gamma pre puberty 5-15 gamma puberty 10-20 gamma adults

8, 5, 4, 2 are the case numbers from the original paper (1963). The first column in each titrated hormone metabolites corresponds to the preoperative finding, whereas the second column shows the findings five years after the operation.

On the other hand in one case in which a small piece of tumor rest persisted the hormone metabolites showed no improvement even in the last control in spite of the same hormone therapy.

#### MATERIAL AND METHOD

Seven of the eight cases of nasopharyngeal angiofibroma which were presented in 1963 were followed up and in only one of them was a piece of tumor rest found in the right corner of the nasopharynx.

The last control, of four cases out of seven was made in 1968. In these cases hormone metabolites were titrated in 24 hour collected urines (the results are given in Table 1). The excretion levels were almost normal in the tumor free cases, whereas in the single tumor-carrying case the result was still abnormal.

For the titration of hormone metabolites we used again the following methods:

1. Urinary 17 ketosteroids were determined by the method of Javie, Crisp & Scholler.
2. 17 OH (17-21 dihydroxy - 20 ketosteroid) was also determined by the Scholler, Busigny & Javie method.
3. Folliculin determination was made by the method of Javie and his co-workers.

#### CONCLUSION

1. Nasopharyngeal angiofibroma is a pathology of the male.
2. In the treatment of this tumor surgical removal is essential. We also think that additional Testoviron therapy may be helpful.

3 Following total extirpation of the tumor and additional hormone therapy hormone excretions gradually returned to normal in three patients.

4 One of the seven cases had a small tumor rest, and his hormone balance did not return to normal in spite of hormone therapy

## RESUME

Sept cas d'angio-fibromes du naso-pharynx furent discuté au congrès de 1963 du point de vue endocrinologique. Quatre de ces 7 cas, qui purent être suivi pendant 3 ans, montrent à la dernière investigation un taux des métabolites presque normal dans le titrage de sujets guéri de leurs tumeurs, tandis que les cas encore porteurs d tumeurs restent comme au début

## ZUSAMMENFASSUNG

Beim Kongress in 1963 wurden 7 Angio-Fibrome Fälle des Naso-Pharynx am end krin logischen Standpunkt us erörtert. Bei 4 von diesen 7 Fällen die 3 Jahre lang nachuntersucht werden konnten wiesen sich die Metabolitenwerte der letzten Hormontitration an tumorfreien Fällen annähernd normal, an tumorhaltigen Fällen dagegen blieben si fast unverändert

## REFERENCES

- Act, A. R. T 1956 The nasopharyngeal fibroma and its treatment. *Arch. Otolaryng* (Chic.) 61 4, 451
- HENDARY M 1954 Hypothalamus et hypophyse *Gynec. (Par)* 53 8, 855
- HOMI, F and WELLS, W A. 1961 Extra nasopharyngeal juvenile angiofibroma *Ann. Otol.* 70 1 164.
- HI AL, A. 1959: Nasopharyngeal angiofibroma *Act Otolaryng* (Stockh.) 5 ppl. 158
- KARA S., KATTACIOGLU S and ENOZONO, O 1963 Significance of hormones in the pathogenesis of nasopharyngeal angiofibroma *Act Otolaryng* (Stockh.) 56 262.
- J ILL, M. F 1961 *Analyses des stéroïdes horm sexuel.* Masson, Paris.
- J ILL, M. F SCHALLER, M HENOT, M., and MET S., 1959 Phénolstéroïdes rinaux. Méthode de dosage. *Clin Chim Acta*, 4 276
- J ILL, M. F and SCHALLER, G., 1958 *Précis de chim physiologique et endocrinologique* Masson, Paris.
- MARTINO, R., and BOUCHER, J 1961 Fibrome Nasopharyngien. *Les maladies pharynx* M son, Paris.
- MARTIN, IL, ELLISON, H. E., and ARZEE, J 1948 Juvenile nasopharyngeal angiofibroma. *Ann. Surg* 127 3, 813.
- OSMON, D A 1959: The so-called juvenile angio-fibroma of the nasopharynx. *J Laryng* 73 8, 293
- SCHIRY M 1959 Juvenile nasopharyngeal angiofibroma. A theory of pathogenesis. *Laryngoscope* 69 901
- THOMAS, F W 1953 Benign tumors of the nasopharynx. *Diseases of the throat nose and ear* p. 272. London.
- WACKENHART, C. A 1957 Troubles endocriniens et électro-encéphalographiques dans le fibrome hémorragique de la puberté *J n. Otolaryng* (P ) 7 845
- WILL, C. D and DODD, J A., 1960- Angiofibroma of nasopharynx. *J Laryng* 74 8
- S. Karatag M.D Elmel Fab Sek. 2  
Stell. Istanbul I, Turkey

## DISCUSSION

*R Albrecht* Hormonanalysen beim Nasenrachenfibrom sind äusserst schwierig, weil es sich um Personen zwischen 10–20 Jahren handelt. In diesem Alter finden wir normalerweise grosse Schwankungen der Hormonausscheidung und ihrer Metaboliten. Deshalb ist es uns bisher nicht gelungen für das Nasenrachenfibrom signifikante Abweichungen nachzuweisen. Bei einer Beobachtung über 5 Jahre muss man damit rechnen, dass sich die hormonale Situation bei diesen Personen stabilisiert. Ich habe deshalb auch Hr. Karatay die Frage gestellt, inwieweit seine Befunde tatsächlich spezifisch auf das Nasenrachenfibrom zu beziehen sind oder auch Ausdruck des veränderten Alters der Patienten sein könnten.

*T Palva* You have suggested that the hormonal level of 17 ketosteroids is lowered by the presence of tumours and that there is a slow return to normal after the tumour has been removed. I should like to know whether or not you have observed any differences in hormone levels in varying sizes of tumour. If the tumour really affects the hormonal balance then the effect of large tumours should be much greater than that of a small tumour. Also when a large tumour is removed and a small recurrence is seen later, is there a difference in the hormone levels pre-operatively and at the time when the recurrence is seen?

*M Portmann* Je voudrais poser 2 questions. (1) Le traitement par hormonothérapie peut-il éviter le traitement chirurgical et quels sont à son avis le rapport de l'hormonothérapie et de la chirurgie dans le traitement des angiofibromes du naso-pharynx? (2) A-t-il vu des angiofibromes guéris uniquement par l'hormonothérapie?

*S Karatay (Reply)* to Mrs Albrecht. In nasopharyngeal angiofibroma we find peculiar features in hormone metabolite titration. Although hormone excretions are not fixed levels in all life, we still know that for normal persons there are some age groups, such as prepuberty age where folliculin is about 0–7 gamma, but in adults it is 10–25 gamma. In our cases we always found folliculin very high, an average of 30 gamma in smaller age-groups than the normal.

To Mr Palva. You are quite right that the finding of changes in titration of hormone metabolites depends upon the size of the tumour. Besides, it should be added that the age of the patient also is very important. If the patient belongs to the smaller age group the tumour is larger and the result to be obtained needs a longer period. Also the tumour pieces left behind have the same influence, which means that if the mass is small the hormone metabolites are on a better level, or vice versa.

To Mr Portmann. We believe that the hormone therapy before the operation reduces the hemorrhage during the operation. But we do not believe that only hormone therapy will reduce the size of the tumour completely, that is why we say that operation is essential. Postoperative hormone therapy will give a chance to the patient to reduce imbalanced androgen-estrogen to normal doses of male hormone, because we are afraid to inhibit the normal endocrine functions.

# A CLINICAL EXPERIMENTAL INVESTIGATION OF THE RATE OF CELL PROLIFERATION IN HUMAN MALIGNANT TUMOURS

S. B. REFRUM *et al.* and P. BERDAL

*From the Institute of Pathology and the Department of Otolaryngology Rikshospitalet, University of Oslo Oslo Norway*

The investigation is based upon mitotic counts in malignant tumours following injection of a mitotic inhibitor. Between the clinical growth and the cell proliferation there is, however, a considerable discrepancy which can only be explained by a very substantial cell loss. The cell loss is discussed.

In the tissue under observation all growth is caused by cell proliferation or cell division. The growth of a malignant tumour is also dependent on cell proliferation, but only scattered information has been available about cell proliferation in malignant tumours in man. The aim of this investigation has been to measure cell proliferation in malignant tumours in man compared to cell proliferation in normal tissues.

In growing tissues and tumours there is an increase in size, i.e. a positive growth, but in the adult organism there is no increase in size, i.e. a steady state. Therefore there must be a cell loss keeping pace with cell proliferation. The growth can in general be expressed as the net increase of cell proliferation over cell loss.

$$G = P - L \quad (\text{Evensen \& Iversen, 1962})$$

The clinical growth of a tumour can be measured by repeated X-ray examinations of primary or metastatic lung tumours over a prolonged period of time, or by direct measurement of superficial tumours. The growth is usually measured as the time needed to double the mass, the so-called "doubling time" (Collins *et al.*, 1958). This doubling time has been found to be remarkably constant in one and the same tumour indicating that growth is exponential, i.e. doubling its size at constant intervals (Breur 1966). Between different tumours, however, there is great variation, with doubling times of only 6-8 days in fast-growing tumours to 300 days or more in slow-growing tumours (Steel & Lamerton, 1966).

The rate of cell proliferation can be measured by stathmokinetic methods. Drugs such as vincoblastine or colchicine and its derivatives, so-called spindle poisons, destroy the spindle apparatus of the mitoses. In so doing, they prevent the chromosomes separating and dividing cells are arrested in

## DISCUSSION

R Albrecht Hormonanalysen beim Nasenrachenfibrom sind äusserst schwierig, weil es sich um Personen zwischen 10-20 Jahren handelt. In diesem Alter finden wir normalerweise grosse Schwankungen der Hormonausscheidung und ihrer Metaboliten. Deshalb ist es uns bisher nicht gelungen für das Nasenrachenfibrom signifikante Abweichungen nachzuweisen. Bei einer Beobachtung über 5 Jahre muss man damit rechnen, dass sich die hormonale Situation bei diesen Personen stabilisiert. Ich habe deshalb und Hr Karatay die Frage gestellt, in wie weit seine Befunde tatsächlich spezifisch auf das Nasenrachenfibrom zu beziehen sind oder auch Ausdruck des veränderten Alters der Patienten sein könnten.

T Palva You have suggested that the hormonal level of 17-ketosteroids is lowered by the presence of tumours and that there is a slow return to normal after the tumour has been removed. I should like to know whether or not you have observed any differences in hormone levels in varying sizes of tumour. If the tumour really affects the hormonal balance then the effect of large tumours should be much greater than that of a small tumour. Also, when a large tumour is removed and a small recurrence is seen later, is there a difference in the hormone levels pre-operatively and at the time when the recurrence is seen?

M Portmann Je voudrais poser 2 questions. (1) Le traitement par hormonothérapie peut-il éviter le traitement chirurgical et quels sont à son avis le rapport de l'hormonothérapie et de la chirurgie dans le traitement des angiofibromes du naso-pharynx. (2) A-t-il vu des angiofibromes guéris uniquement par l'hormonothérapie.

S Karatay (Reply) to Mrs Albrecht In nasopharyngeal angiofibroma we find peculiar features in hormone metabolite titration. Although hormone excretions are not fixed levels in all life, we still know that for normal persons there are some age groups, such as prepuberty age, where folliculin is about 0-7 gamma, but in adults it is 10-25 gamma. In our cases we always found folliculin very high, an average of 30 gamma in smaller age-groups than the normal.

To Mr Palva You are quite right that the finding of changes in titration of hormone metabolites depends upon the size of the tumour. Besides, it should be added that the age of the patient also is very important. If the patient belongs to the smaller age group the tumour is larger and the result to be obtained needs a longer period. Also the tumour pieces left behind have the same influence, which means that if the mass is small the hormone metabolites are on a better level, or vice versa.

To Mr Portmann We believe that the hormone therapy before the operation reduces the hemorrhage during the operation. But we do not believe that only hormone therapy will reduce the size of the tumour completely, that is why we say that operation is essential. Postoperative hormone therapy will give a chance to the patient to reduce imbalanced androgen-estrogen to normal doses of male hormone because we are afraid to inhibit the normal endocrine functions.

TABLE 2. Tumour localisation

	No. of patients
Larynx	32
Maxilla	13
Oral cavity	11
Other sites	5

nearly double this amount of cells divide. The reason may be that the wear and tear of the epithelial cells in the mouth is much greater than in the larynx. In order to replace this greater cell loss a greater cell proliferation is necessary. In mucous and serous glands, which are far less exposed to mechanical trauma, the rate of cell proliferation was about one tenth of the cell proliferation found in thin epithelium.

In atypical epithelium and in carcinomas the rate of cell proliferation was somewhat higher than in hyperplastic epithelium, but the difference was not pronounced (carcinomas 11.45 thick epithelium, mouth and tongue 10).

The distribution of cell proliferation found in different areas of epithelium and tumours is shown in Fig. 1. The abscissa shows the rate of cell proliferation and the ordinate the number of biopsies or tumour areas counted. We can here observe three points of interest.

1. In all tissues there was a wide range in the rate of cell proliferation, most pronounced in atypical epithelium and in carcinomas where there was a 40-70-fold increase in the rate of cell proliferation from slow to fast proliferating areas.

2. Although the average rate of cell proliferation in malignant tumours was somewhat higher than in normal and hyperplastic epithelium, many

TABLE 3. Rate and range of cell proliferation in different epithelium and in tumours

	Rate and range of cell proliferation per 1000 cells/h
Mucous and serous glands	0.5 (0.3-1.0)
Columnar and thin epidermoid epithelium (larynx/trachea)	4.8 (0.2-14.0)
Thick or hyperplastic epidermoid epithelium (mouth/tongue)	10.0 (3.0-21.0)
Atypical epithelium	12.8 (0.8-21.0)
Carcinomas	11.45 (0.6-22.0)

mitosis without any influence on the number of cells about to divide (Eigsti & Dustin, 1955). A biopsy taken after a colchicine injection will therefore show an increased number of cells in mitosis in the tissue. If the relative number of cells in mitosis are counted and the time between the colchicine injection and the biopsy is known, the number of cells dividing per unit of time can be calculated, i.e. the rate of cell proliferation

$$P = \frac{\text{No. of arrested mitoses}}{\text{Time between colchicine inj. and biopsy}}$$

In contrast to clinical growth and cell proliferation, there is today no known method of measuring cell loss in a tumour directly. Cell loss must therefore be calculated as the discrepancy between growth calculated on the basis of cell proliferation and actual clinical growth of the tumour.

### MATERIAL AND METHOD

During the last 3 years we have given an i.v. injection of 10 mg demecolcine (Colcemid, Ciba), a derivative of colchicine but considerably less toxic to nearly every patient treated for malignancy in the ENT department of Rikshospitalet. We have not observed any adverse effects.

Most of the patients had more or less differentiated epidermoid carcinomas (Table 1) mostly located in the larynx, the maxilla, and the oral cavity (Table 2). The average age was 59 years.

Usually 2-6 pieces of the tumour were taken from each patient and in each tumour area an average of about 5000 tumour cells were counted. An average of about 23 000 tumour cells were thus counted in each patient. As many of the biopsies were covered by normal, hyperplastic, or atypical epithelium the number of arrested mitoses were also counted in these tissues. The results were expressed as the number of mitoses dividing per 1000 cells per hour.

### RESULTS

In the normal squamous and cylindrical epithelium of the larynx and trachea the rate of cell proliferation was about five cells per 1000 cells per hour (Table 3) whereas in the hyperplastic epithelium of the oral cavity

TABLE 1 *Tumour histology*

	No. of patients
Epidermoid carcinomas	46
Salivary gland carcinomas	5
Undifferentiated tumours	5
Other tumours	5

and corrected for an exponential growth pattern, the doubling time of the malignant tumours investigated should vary between 0.8 and 10.7 days, with an average of only 2 days ( $G = \ln P$ )

Although the doubling time of maxillary and laryngeal tumours is unknown, we have assumed that this doubling time does not differ essentially from the doubling time of primary or metastatic lung tumours which is found to be about 2-3 months on average (Breur 1966 Steel & Lamerton, 1966)

The discrepancy between clinically measured growth and doubling time calculated on the basis of cell proliferation can, therefore, only be due to cell loss. In this material cell loss can be calculated as 95% or more of cell renewal in the tumour. How this cell loss is brought about is unknown. In theory however the following may be possible:

1. Cell loss from tumour surfaces or to blood vessels and lymphatics.
2. Necrosis due to circulatory failure (infarction) or to toxic substances.
3. Non-viable tumour cells (lethal tumour cells) due to insufficient enzyme systems, etc.
4. Immunological cytotoxicity due to
  - (a) Humoral or cell bound specific or unspecific antibodies.
  - (b) The organism's normal defence mechanism against damaged or altered cells.

Which of these processes may take place in a particular tumour is unknown. Some case histories, moreover, indicate that some tumours have a cell loss considerably less than calculated above, as these tumours have an exceptionally fast clinical growth without any particular fast cell proliferation. Why cell loss may fall in some patients is unknown.

Neoplastic growth must be looked upon as, on the one hand, caused by an uncontrolled cell proliferation, regardless of how fast or slow it is, and on the other hand by a very substantial cell loss.

## RESUME

L'analyse se base sur la régression des mitoses dans les tumeurs après l'injection d'un inhibiteur mitotique. Une différence considérable entre l'accroissement clinique des tumeurs et la prolifération cellulaire est démontrée. Cette différence est expliquée par un pert cellulaire des tumeurs.

## ZUSAMMENFASSUNG

Die Untersuchungen sind auf Zählungen der Mitosen nach Injektion einer mitosenhemmenden Substanz basiert. Ein bedeutender Unterschied zwischen dem klinischen Zuwachs des Tumors und der zellulären Proliferation ist gefunden. Dieser Unterschied wird durch einen Verlust von Zellen erklärt.



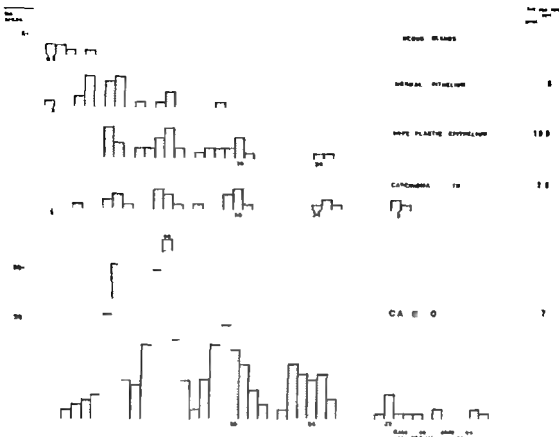


FIG. 1 Distribution of different rates found in different areas counted in carcinomas and in atypical and normal epithelium.

malignant tumours had a rate of cell proliferation equal to or even lower than in normal epithelium

This is of particular interest as the use of many of the cytostatics is based on the assumption of a high proliferation rate. If the action of the cytostatics is solely due to its action on fast proliferating tissues, we can not expect to damage or kill tumour cells without damage to normal cells.

3. In tumours where more than one area of tumour cells was counted, different rates were often found in different areas counted. This is in good agreement with the clinical observation that a tumour does not always grow with equal speed in all areas.

### Cell Proliferation and Cell Loss

As formerly stated, there is today no method of measuring cell loss directly and cell loss must be calculated as the discrepancy between rate of cell proliferation and the actual clinical growth of the tumour ( $G = P - L$ ).

The doubling time can easily be calculated on the basis of cell proliferation, provided there is no cell loss. If 10 cells divide per 1000 cells per hour then all the cells would have divided in 100 hours. Calculated in this way

and corrected for an exponential growth pattern, the doubling time of the malignant tumours investigated should vary between 0.8 and 10.7 days, with an average of only 2 days ( $G = \ln^2/P$ )

Although the doubling time of maxillary and laryngeal tumours is unknown, we have assumed that this doubling time does not differ essentially from the doubling time of primary or metastatic lung tumours which is found to be about 2-3 months on average (Breur 1965 Steel & Lamerton, 1966)

The discrepancy between clinically measured growth and doubling time calculated on the basis of cell proliferation can, therefore, only be due to cell loss. In this material cell loss can be calculated as 95% or more of cell renewal in the tumour. How this cell loss is brought about is unknown. In theory however the following may be possible

- 1 Cell loss from tumour surfaces or to blood vessels and lymphatics.
- 2 Necrosis due to circulatory failure (infarction) or to toxic substances.
- 3 Non viable tumour cells (lethal tumour cells) due to insufficient enzyme systems, etc.
- 4 Immunological cytotoxicity due to
  - (a) Humoral or cell bound specific or unspecific antibodies.
  - (b) The organism's normal defence mechanism against damaged or altered cells

Which of these processes may take place in a particular tumour is unknown. Some case histories, moreover, indicate that some tumours have a cell loss considerably less than calculated above, as these tumours have an exceptionally fast clinical growth without any particular fast cell proliferation. Why cell loss may fall in some patients is unknown

Neoplastic growth must be looked upon as, on the one hand, caused by an uncontrolled cell proliferation, regardless of how fast or slow it is, and on the other hand by a very substantial cell loss.

## RÉSUMÉ

L'investigation se base sur la résultante des mitoses dans les tumeurs après l'injection d'un inhibiteur mitotique. Une différence considérable entre l'accroissement réel des tumeurs et la prolifération cellulaire est démontrée. Cette différence est expliquée par une perte cellulaire des tumeurs.

## ZUSAMMENFASSUNG

Die Untersuchungen sind auf Zählungen der Mitosen nach Injektion einer mitosenhemmenden Substanz basiert. Ein bedeutender Unterschied zwischen dem klinischen Zuwachs des Tumors und der zellulären Proliferation ist gefunden. Dieser Unterschied wird durch einen Verlust an Zellen erklärt.

## REFERENCES

- BREUR, K. 1966: Growth rate and radiosensitivity of human tumours. I Growth rate of human tumours *Europ J Cancer* 2 157
- COLLINS, V. P. LOEFFLER, H. and TIVY H. 1956 Observations on growth rates of human tumours *Amer J Roentgenol* 76 988
- FISCH, O. J. and DUSTIN P. JR., 1955 *C. Ichicine in agriculture medicine biology and chemistry* The Iowa College Press, Ames, Iowa.
- EVENMAN, A. and IVERSEN, O. H. 1962: Rat of cell proliferation in a mouse squamous cell carcinoma *Nature* 196 383.
- STEEL, G. G. and LAVERTON L. F. 1966 The growth rate of human tumours, *Brit. J Cancer* 20 74

P. Berd I. M.D.  
Dept. of Otolaryngology  
University of Oslo  
Oslo, Norway

## DISCUSSION

A. H. Vosteen: Haben Sie auch andere Untersuchungen für Bestimmung der Malignität (z. B. Chromosomenanalyse, D\N-Messungen etc.) durchgeführt und mit der Verdoppelungszeit verglichen? Wir haben versucht mit der Messung der Verdoppelungszeit während der R<sub>0</sub>-Bestrahlung auch die Radiosensibilität eine Verdoppelungszeit von 6 Tagen nach 1000 R von 10 Tagen und nach 3000 R von 100 Tagen. Ich möchte deshalb fragen ob Herr Refsum ähnliche Erfahrungen gemacht hat.

S. B. Refsum (Antwort) an Hr. Vosteen: Wir haben Chromosomenanalyse in vielen Tumoren durchgeführt, aber wir haben noch nicht die Resultate von der Chromosomenanalyse mit der Zellproliferation verglichen. Wir haben auch die Zellproliferation in R<sub>0</sub>-bestrahlten Tumoren untersucht. Die durchschnittliche Zellproliferation nach R<sub>0</sub>-Bestrahlung war etwas weniger als die Zellproliferation in nicht R<sub>0</sub>-bestrahlten Tumoren. Der Zeitraum zwischen dem Anfang der Symptome und der Behandlung war jedoch einige Monate länger in den R<sub>0</sub>-bestrahlten Tumoren als in den nicht bestrahlten Fällen. Es ist deshalb möglich, dass die langsamere Zellproliferation in den R<sub>0</sub>-bestrahlten Tumoren mit einer Auswahl von den R<sub>0</sub>-bestrahlten Tumoren zusammenhängt und nicht mit einer Depression der Zellproliferation von R<sub>0</sub>-Bestrahlung. Der Zeitraum zwischen Bestrahlung und Rezidiv war kürzer in Fällen mit einer schnellen Zellproliferation als in Fällen mit langsamerer Zellproliferation.

Der Zeitraum zwischen der Bestrahlung und der Untersuchung der Zellproliferation war einige Tage bis mehrere Jahre. Wir haben keine Erfahrung mit dem Verhältnis der Zellproliferation vorher und kurz nach einer R<sub>0</sub>-Bestrahlung.

## QUELQUES ASPECTS PARTICULIERS DE LA MORPHOLOGIE PAROTIDIENNE NORMALE ET PATHOLOGIQUE

F. BRAUNETTI

*Clinique Oto-rhino-laryngologique de l'Université de Torino Torino Italie*

Quelques caractéristiques singulières de la submicroscopie de la parotide humaine normale et quelques aspects peu communs de la pathologie néoplasique parotidienne sont présentés. Les données de microscopie optique sont comparées avec celles de la microscopie électronique.

Particulièrement riche d'études et de documentations, la littérature sur les tumeurs parotidiennes a eu comme objet de ses recherches le polymorphisme histologique et cytostructuel des tumeurs épithéliales salivaires et les hypothèses de leur dérivation histogénétique.

Classiquement on confirme spécialement pour la parotide, la nette prédominance (incidence moyenne du 0%) des ainsi dites tumeurs mixtes qui correspondent au type des épithéliomes remaniés des AA français, dont tout le monde connaît la lenteur de croissance et les poussées évolutives, en rapport aussi avec la vie génitale (retour des règles après parotidectomie chez la femme, adénome parotidien et parotidomégalie chez l'homme dans la vieillesse).

Au point de vue chirurgical en outre, est bien déterminé le rôle aggravant des interventions successives (Dargent) et l'inutilité des interventions partielles.

Dans l'ordre de fréquence décroissante (à peu près le 10% des néoplasies salivaires) viient les tumeurs muco-épidermoïdales, décrites pour la première fois en 1940 par Skorpil et successivement illustrées d'une façon systématique par Stewart, Forte et Becker. Ces tumeurs dériveraient de cellules indifférenciées qui se seraient détachées des premiers bourgeons des ducts glandulaires. Pour ce type de néoplasme on a aussi constaté une grande prédominance de localisation au niveau de la parotide (80% des cas).

La morphologie de cette tumeur est variable d'une zone à l'autre. En effet à côté de zones où les cellules sont claires avec noyau placé sur la membrane cellulaire il y a des zones où les cellules ont un cytoplasme dense et des contours non bien définis. Dans ces zones les cellules épithéliales nous rappellent, par leurs caractères, les éléments de l'épithélium de revêtement, pouvant avoir en effet une disposition stratifiée ou bien rester réunies en un compact autour des zones constituées entièrement par des cellules à cytoplasme clair (Figs. 1, 2 et 3).

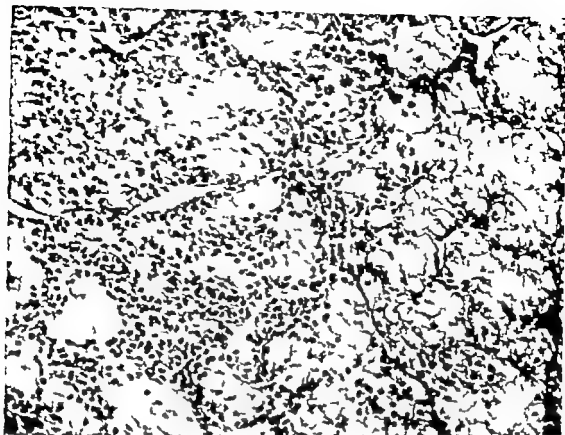


FIG. 1. Hém. éos. 70. Tumeur muco-épidermoïdale. Vision d'ensemble : la de cellules vasculaires et de zones d'aspect solide à cytoplasme dense (2 parties tumorales : partie muqueuse et partie épidermoïdale).

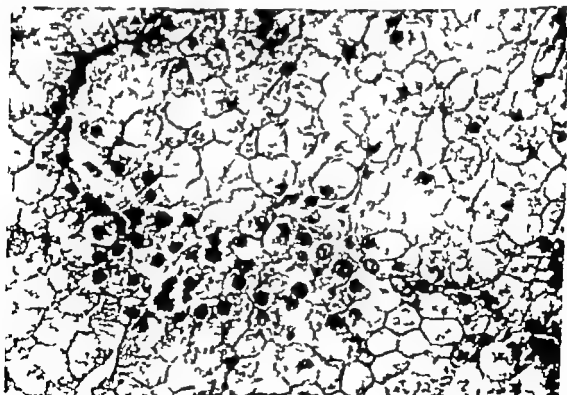


FIG. 2. Hém. éos. 375. Tumeur muco-épidermoïdale : Cellules écumeuses et lésées avec contours de cellules nucléées (partie muqueuse).

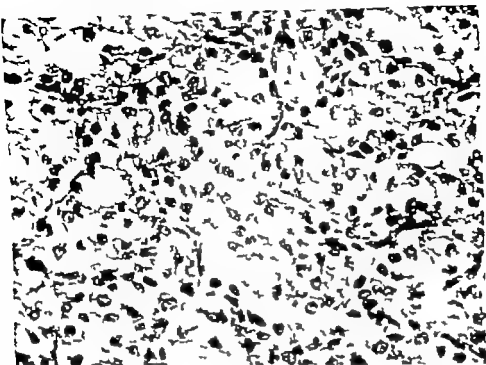


FIG. 3. Ilém éos. 373 T meur mées-épidermoïdal. Cell les à cytoplasme dense grossier les da lesquelles les noy se d pose t ta hyperchromatiques qui rappellent la morphologie des cellules de la couche basale de l'épithélium muqueux. P contre les cellules et les noy sont placés les comme dans la couche muqueuse d'épithélium (partie épidermoïdal de la t meur).

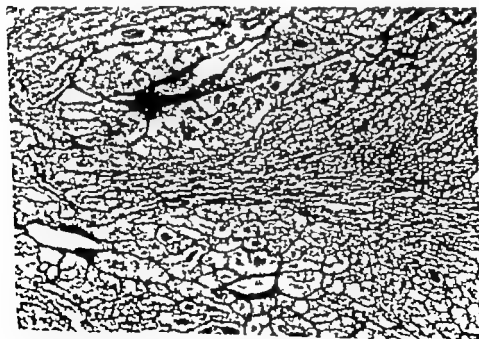


FIG. 4. McM au "0; Adénome à cellules acineuses. Réseau conjonctif qui divise des zones épithéliales cordonnées de type "esculaire".

Une incidence presque analogue aux tumeurs mucoépidermoïdales aurait le cylindrome de Billroth (1859) qui a été en suite défini par Foote & Frazer (1954) comme « adenoid cystic carcinoma »

L'organisation architecturale et cytomorphologique du cylindrome, analogue aux tumeurs mixtes, serait représentée par des formes canaliculaires, végétantes et lobulées ou par des formes acineuses et alvéolaires à cellules soit cylindro-cubiques éosinophiles, basophiles ou claires, soit fusiformes ou étoilé et enfin par des formes de dérivation qui paraissent dûes à différentes maturations ou dégénération cytotumorales (dégénération mucoside jaline ou mixocondroïde par réaction d'auto-anticorps)

Tous les éléments du carcinome adénocystique correspondent aux différentes structures cellulaires des glandes salivaires (cellule sécrétantes, bordantes et myo-épithéliales des canaux glandulaires)

Un autre type de tumeur parotidienne est l'« adénome à cellules acineuses » qui dériverait des éléments des acini glandulaires

La structure générale est celle d'une tumeur capsulée à tissu condensé et à caractère plurifocal. Les cellules tumorales se trouvent dans des espaces allongés dont la structure est soulignée par l'abondant réseau conjonctival (Figs. 4 et 5)

La bénignité histologique apparente de cette tumeur peut être caractérisée par un décours clinique malin (possibilité de métastases à distance et de métastases lymphatiques régionales) et c'est pour cela qu'on conseille dans ces cas la parotidectomie totale avec évidemment radical du cou

L'adénome oncocyttaire ou oncocytome est caractérisé par des cellules à cytoplasme clair éosinophile avec petit noyau à chromatin épais disposés en longues colonnes avec une nette et bien précise controposition des noyaux en deux files contigües. La parenchyme néoplasique est divisé en lobules de dimensions inégales au centre desquels on peut observer par des phénomènes de nécrose une dissociation des cellules tumorales (Figs. 6 et 7)

L'hystogénèse de l'oncocytome a été attribuée à des cellules salivaires vieillissantes. Au point de vue hystogénétique les cellules de l'oncocytome repètent la morphologie et les caractéristiques de l'onco-cyte décrit en 1931 par Hamperl et identifié comme une cellule salivaire vieillissante (Fig. 8)

Fréquence mineure (4-5%) auraient enfin les adénolymphomes.

Ce type de neoplasie est constitué par des tas de cellules analogues aux éléments des conduits glandulaires, tas qui sont quelques fois réunis en longues colonnes autour de cavités d'amplitude variable. Dans d'autres zones il y a une très riche infiltration d'éléments de la série lymphocytaire qui soulèvent ces colonnes cellulaires et donnent origine à des formations papillaires, qui plongent dans des cavités de substance amorphe (Figs. 9 et 10)

L'hystogénèse la plus probable des cysto-adéno-lymphomes est représentée par la dérivation de l'épithélium des conduits glandulaires sécréteurs.

Sur la base des documentations récentes de la microscopie électronique

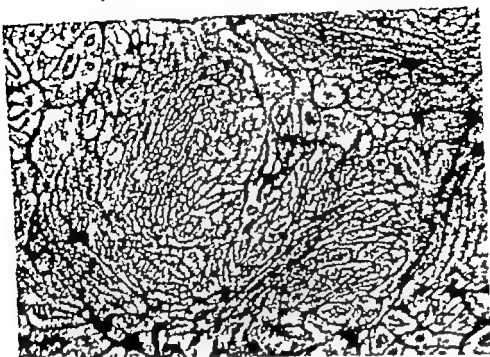


FIG. 5. MALLORY. — Adénome à cellules acineuses. Disposition centrifuge du réticulum conjonctif.

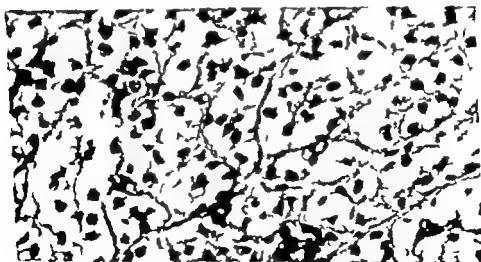


FIG. 6. MALLORY. — Adénome à cellules acineuses. Morphologie typique de la cellule à cell. les acineuses. Cellules claires et cytoplasme vacuolaire. Pseudo-glandulaire, à disposition pseudoglandulaire. Il y a de cell. les claires, et non pas de chromatine qui se trouve presque toujours à la périphérie de la cellule. Il est pas rare de trouver des résidus de canaux glandulaires qui conservent une disposition et une structure plus ou moins régulières.



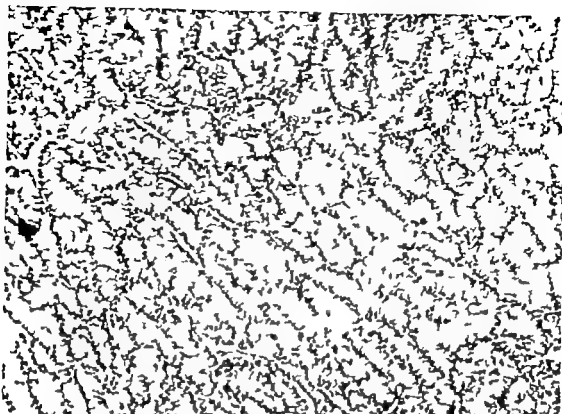


FIG. 7 Hém. éo 70 Adénom oncocyttaire : Disposition à cordon des cellules à cytoplasme granuleux et aspects pseudo-fasciculés qui s'accentuent surtout vers la périphérie avec des travertineux dans les zones plus centrales des lobes tumoraux.

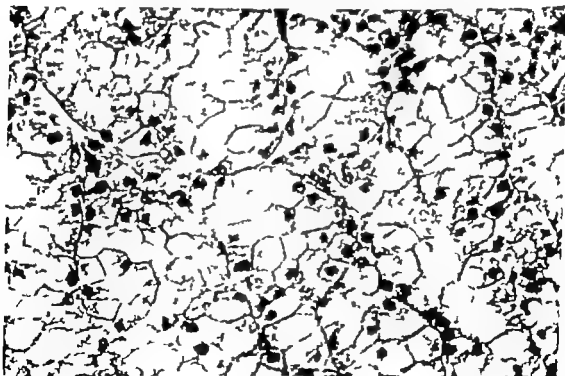


FIG. 8 Hém. 378 Adénom oncocyttaire : Caractéristiques des oncocytes : cellules à cytoplasme granuleux, limites nettes, disposition en cordons et en travertineux, et disposition marginale des noyaux.



FIG. 9. Idem. éos. 70 Adénolymphome. On voit à rubans de cellules épithéliales cylindriques et quelques formations kystiques.

nous nous limiterons maintenant tout d'abord l'architecture et la structure fine des glandes salivaires par rapport aux différentes situations morpho-fonctionnelles liées à l'activité sécrétoire.

Nous rappellerons à ce propos que la microscopie optique a déjà permis de démontrer une diminution des grains spécifiques qui existent dans les cellules sécrétoires pendant les différentes phases de l'activité sécrétoire glandulaire. Pendant la phase de repos il y aurait une augmentation des mitochondries, de la formation de Golgi et de la substance chromophile (Bloom & Fawcett, 1968).

Les recherches ultramicroscopiques ont ultérieurement précisé les caractéristiques des activités sécrétoires et des interactions fonctionnelles intracellulaires représentées par des inflexions de la membrane plasmique du pôle basal, entre lesquelles se tassent des files de mitochondries (Bloom & Fawcett, 1968).

Pour la parotide humaine nous avons examiné l'ultrastructure de l'épithélium au niveau des adénomères, des dômes intercalaires (ou préterminaux) et des dômes striés.



FIG. 10. Hém. eos.  $\times 375$ . Adén. lymphome : épithélium cylindrique soulevé en papilles avec un axe fibroco-jonctival infiltré d'lymphocytes.

Les clichés qui suivent démontrent les caractéristiques de l'épithélium de ces structures (Figs 11, 12, 13, 14, 15).

Enfin dans la dernière photo (Fig. 16) on document l'aspect ultrastructural des éléments du conduit strié. La striature caractéristique de la zone basale de l'épithélium des ducts striés qu'on voit au microscope optique paraît être déterminée, au microscope électronique par des mitochondries tassés entre les inflexions du plasmalemma.

On a ainsi démontré par la microscopie électronique que l'image caractéristiquement striée de la partie basale du ainsi-dit « épithélium bacillaire » est déterminée par des tas de mitochondries intercalés entre les inflexions du plasmalemma.

Cette dernière interprétation, déjà conçue par le passé comme hypothèse par la microscopie optique, confirme la grande variété cytostructurale qui existe entre les différents éléments qui constituent les différentes portions des unités glandulaires de l'organe salivaire.

Ce polymorphisme cytostructural, qui varie selon les différentes conditions fonctionnelles de l'activité sécrétoire salivaire, pourrait, à notre avis, justifier l'extrême variabilité cytomorphologique et histopathologique qu'on observe chez les tumeurs parotidiennes.



FIG. 11 Phot. électronique qui illustre les caractéristiques ultra-microscopiques d'un sécrétory. On observe des cellules où le cytoplasme paraît très dense et des vésicules et des granules. Toutes les cellules contiennent des granules de sécrétion, qui ne sont pas tous parfaitement homogènes, et des formations à opacité électronique variable. Dans la cellule centrale et à microscopie on voit un réseau endoplasmique réticulaire lisse et de nombreux ribosomes libres.



FIG. 12 Z n d cont t cell laire dans un dén mère Entre les il les il u y p une  
 dhérence nt me mal ch que élément est séparé des utres pa espace perméabl  
 aux lectrons, dan lequ l on it d n mbreuses digitation cytopl matiques = form  
 de micro lli



Fig. 13. Phot. électronique dans laquelle on observe une cellule myo-épithéliale interposée entre la membrane basale de l'adénoacrome et une cellule salivaire. Le cytoplasme de cette cellule possède un reticule granulaire endoplasmique abondant et quelques mitochondries qui pénètrent dans une zone perméable électronique, située entre la cellule myo-épithéliale et la cellule salivaire.



FIG. 12 Zone de contact. Ilulire dans un dénomère. Entre les cellules il n'y a pas une adhérence intime, mais chaque élément est séparé des autres par un espace perméable aux électrons, dans lequel on voit de nombreuses digitations cytoplasmiques en forme de microvilli.



Fig. 12. Phot. électronique de laquelle on observe une cellule myo-épithéliale interposée entre membrane basale de l'adénomère et une cellule salivaire. Le ryl pl m de cette cellule possède un réticulum granulaire endoplasmatique abondant et qu'il y a des microvilli qui pénètrent dans une membrane électronique, située entre la cellule myo-épithéliale et la cellule salivaire.



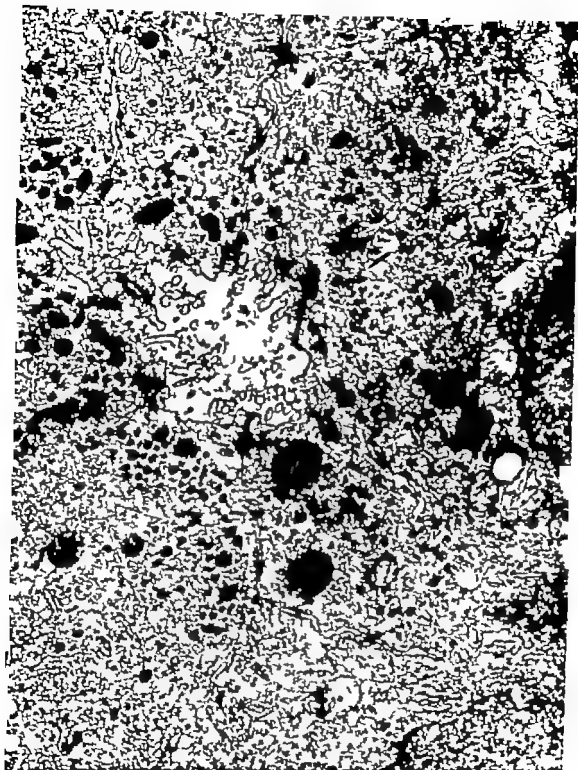


FIG. 14. Caractère submicroscopiques de la première partie d'un d'lit intercalaire près du d'lit mère. On observe nombreuses cellules alvéolaires, qui contiennent plus le plus grand de sécrétion, réunies en îlots par des complexes de jonction à la partie plus superficielle. Ces cellules sont entourées d'une petite membrane perméable aux électrolytes dans laquelle se trouvent des propagés cytoplasmiques, type microfilaments et vers lesquels semblent se diriger les grains de sécrétion.

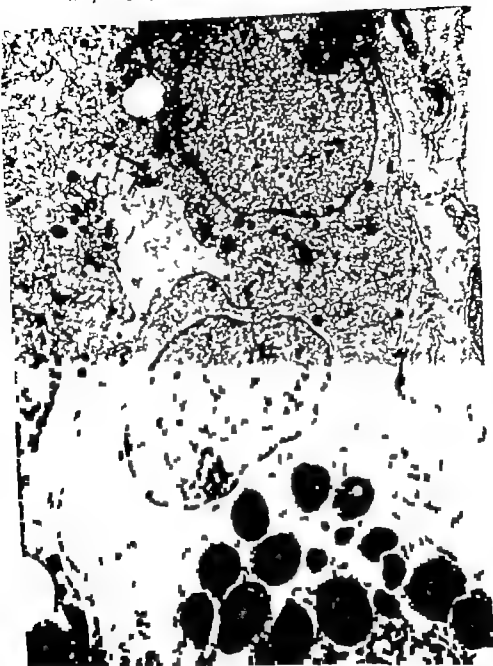


Fig. 13. Caractères submicroscopiques de la partie terminal d'un dotti intercalaire vers le dotti strié. Les cellules qui délimitent le dotti intercalaire contiennent des grains de sécrétion en quantité inférieure à celle qu'on peut remarquer sur la photo précédente.

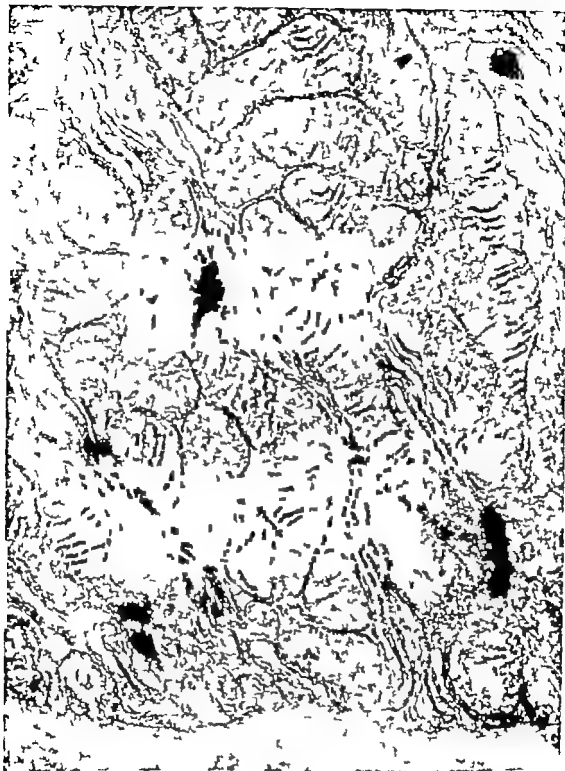


Fig. 16 Morphological ultrastructure of the basal part of an epithelium. The striated structure characteristic of the basal part of the epithelium is visible. The striated structure appears to be determined by the presence of myofibrils. The striated structure is visible in the basal part of the epithelium.

## SUMMARY

Some singular sub-microscopical characteristics of the normal human parotid and some non-common aspects of coplastic parotid pathology are presented. Observation by optic microscope are correlated with those obtained by electron microscopy.

## ZUSAMMENFASSUNG

Verf. stellt ungewöhnliche submikroskopische Aspekte der normalen menschlichen Parotis und einige Bilder der neoplastischen Parotispathologie dar. Die optisch-mikroskopischen Bilder werden mit denen der Elektronenmikroskopie zusammengestellt.

## BIBLIOGRAPHIE

- BROWN, W. et F. WERTZ D. W. 1968 *A textbook of histology* 3e edn. Philadelphia.  
 FOOTE, F. W. et F. UZZO, E. L. 1954 *Tumors of the major salivary glands*. Armed Forces Institute of Pathology Washington.  
 SERRA, J. P. 1964 A classification of major salivary gland lesions. *Arch. Otolaryng.* (Chic.) 80: 322.  
 — 1966 *Head pathology of salivary gland lesions*. Pitman Medical Publishing, London.  
 and J. B. Lippincott, Philadelphia.

*Chirurgie Otolaryngologique*  
 et *Chirurgie de l'oreille*  
 Torino 11 (1)

## DISCUSSION

J. Friedmann: A pathologist I agree that diagnostic difficulties are encountered in this field. The surgeon I not only guided by the pathologist's findings but also at a correct diagnosis. We have been hoping that electron microscopy might provide an additional clue. This has so far been the case only in regard to the adenolymphoma (M. Brunetti has shown an example of this tumour). Light microscopy of the adenolymphoma is so characteristic that one seldom forgets it. The electron microscope has revealed a characteristic crystalline inclusion first described by Shipke from the Sloan Kettering Institute. Two practical questions arise. The first of evidence in this material. The site of the tumours, especially the cribriform, depends on material they were localised mainly in the accessory salivary glands. Differential diagnosis between "benign" and malignant pleomorphic cellular parotid gland neoplasms.

J. Werrath: I want to stress the importance of combining light and electron microscopy in this tumour studies. Mr. Ennoth, Moherger, Hjerthman, and I have studied parotid tumours with light and electron microscopy during the last few years. We have found that it is not only possible to achieve a better and more exact diagram in some cases, but also to get some information about the prognosis of the tumour by the use of this combined study.

It is found that the compact type of adenoid cystic carcinoma has a worse prognosis than the cribriform type. With electron microscopy we can prove that the prognosis is related to the amount of hyaline basilar membrane-like material produced by the tumour. High production of this substance, as in the cribriform type, is related to better prognosis than that of tumours with a lesser production of the basement membrane-like substance.

F. Brunetti (Réponse): À M. Friedmann et M. Werrath. Ces recherches submicroscopiques ont été faites premièrement dans la parotide normale.

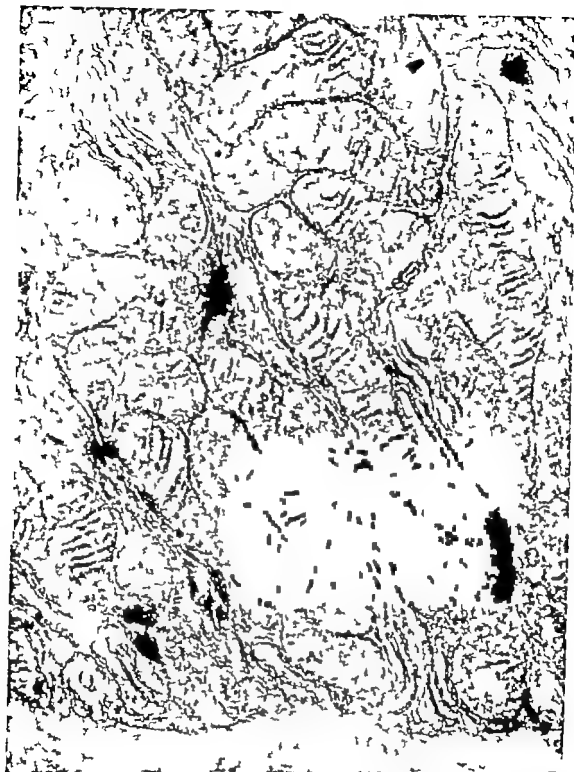


FIG. 16 Morphologie submicroscopique de l'épithélium buccal d'un diti strié. La structure caractéristique de la zone basale de l'épithélium de diti striés qui n'est pas visible au microscope optique paraît être déterminée au microscope électronique par des modifications entre les inflexions de plasmalemma.

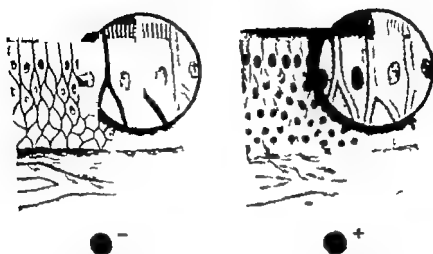


Abb. 1. Unterschiedliches Eindringen von leicht nach dissoziierten kristalloiden Farbstoffen (Fluorochromen) in die Schleimhaut. Links: Ausdrückte Farbstoff-Ionen werden orangefarben (interzell.) durch Epithel transportiert. Rechts: Bei katodischen Farbstoff-Ionen erfolgt der Transport orangefarben (interzell.)

Mechanismus des Resorptionsvorganges, sie erlaubten jedoch keine quantitativen Aussagen. Um solche zu erhalten, müssen weitere Methoden herangezogen werden. Theoretisch kommen für diesen Zweck in Frage:

(a) Die Messung pharmakologischer Effekte während und nach Resorption entsprechender Wirkstoffe. Diese Möglichkeit wurde z. B. von Tonnhofer *et al.* (1933) mit Hyoseamin und von Bofik (1963) mit Pilocarpin eingesetzt. Die hiermit erhaltenen Ergebnisse sind naturgemäß sehr grob.

(b) Die indirekte Messung radioaktiv markierter über die Nasenschleimhaut resorbierter Substanzen in den Ausscheidungen, wie sie z. B. Brenninger & Feine (1968) mit dem Nachweis von  $C^{14}$ - $CO_2$  in der Exhalationsluft kürzlich publiziert hat. Auch bei diesem Prinzip sind nur verhältnismäßig grobe Relativzahlen zu gewinnen, ganz abgesehen davon, daß die bisher angewandten Methoden vermutlich Fehlerquellen enthalten.

(c) Der kombinierte direkte quantitative Nachweis einer mit Nukliden markierten resorbierten Substanz am Orte der Resorption im Blut, im Gesamtorganismus und gegebenenfalls in den Ausscheidungen. Diese Kombination verschiedener Meßverfahren gestaltet nicht nur sehr viel exaktere Angaben über Art und Grad der Resorption eines Stoffes, sie erlaubt auch die Prüfung und den Nachweis von Stoffen unterschiedlichen Molekulargewichtes, sowie unterschiedlicher chemischer Verbindungen und Komplexe in systematischen Versuchsserien.

(d) Prinzipiell kann diese Methodenkombination noch ergänzt werden

## ZUR PROBLEMATIK DER RESORPTIONSMESSUNG AN DER NASENSCHLEIMHAUT

H H NAUMANN W H NAUMANN M MÜNZEL und K OEFF

*Aus der HNO-Klinik der Freien Universität Berlin Berlin Deutschland*

Verschiedene Methoden zur Messung der Resorptionsleistung der Nasen Schleimhaut werden kritisch diskutiert. Eigene Meßergebnisse mit  $J^{125}$  markierten Substanzen (Jod Hippuran, Lysozym, Human Albumin) sowie kolloidalem  $Au^{198}$  werden mitgeteilt wobei verschiedene Meßverfahren kombiniert werden (Aktivitätsmessung über der Resorptionsstelle im Blut, in den Ausscheidungen sowie als Scintigramm) — Es zeigt sich, daß die gemessenen Aktivitäts Werte im Blut nicht ohne weiteres gleichzusetzen sind mit der resorbierten Menge eines markierten Komplexes (z B Human Albumin) Es gelang der Nachweis, daß von einer im Blut gemessenen Gesamtkaktivität bei den Albumin Resorptionsversuchen nur etwa 1 auf Albuminkomplex / auf freies Nuklid oder Albumin Bruchstücke entfallen Die möglichen Ursachen werden diskutiert und einige weitere Fragen gestellt

Im intravitalmikroskopischen Experiment hatte einer von uns (H H Naumann, 1958) mit Hilfe der Fluoreszenz Mikroskopie bereits früher folgendes feststellen können

1 Wasserlösliche kristalloide Lösungen werden sehr rasch von einer normalen Nasenmukosa aufgenommen Die Übergangsgeschwindigkeit ins Blut kommt unter günstigen Bedingungen der einer i.v. Injektion sehr nahe

2 Je nach Art der elektrischen Dissociation der Fluorochrome ist der Resorptionsmechanismus prinzipiell unterschiedlich (Abb 1) Kathodische Ionen ( ) erfahren bei relativ langsamer Ausbreitung auf der Schleimhaut oberfläche eine *rasche* Aufnahme in die Mukosa selbst der Weitertransport erfolgt vorwiegend von Zelle zu Zelle, der Abtransport über die Gefäße ist relativ langsam Anodische Ionen ( ) hingegen breiten sich auf der Oberfläche schnell und weit aus, sie dringen etwas langsamer in die Mukosa ein und zwar vorwiegend zwischen den Zellen, ihr Abtransport über die Gefäße ist dann jedoch relativ rasch Nicht dissoziierte, neutrale Fluorochrome liegen in ihrem Verhalten zwischen dem der anionischen und dem der kationischen

3 Es zeigte sich ferner daß kolloidale Farbstoffe sehr viel langsamer als Kristalloide von der Schleimhaut aufgenommen werden

Diese und eine Anzahl weiterer intravitalmikroskopisch gewonnener Beobachtungen (H H Naumann, 1969) ergaben zwar *qualitative* Einblicke in den



Abb. 3

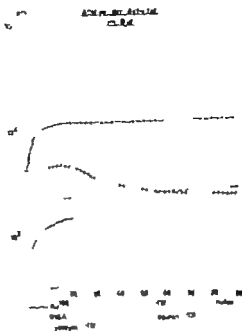


Abb. 4

gewonnenen Serum wurde dann ebenfalls mit einem Thallium-aktivierten Natrium-Jodid-Kristall (Bohrloch 2 × 2 inches) die Aktivität gemessen. Abb. 4 zeigt einige auf diese Weise gefundene Werte. Auf ein besonderes Problem bei diesen Ergebnissen kommen wir später noch zu sprechen.

3. Mit einem Picker Magna-Photoscanner führten wir Ganzkörper-Scintillogramme an den Versuchstieren durch. Wir verglichen dabei das Scintillogramm am Ende eines Resorptionsversuches mit Human-Albumin (nach 90 min.) mit einem Scintillogramm nach einer ersten Spülung der Nase mit 100 cm<sup>3</sup> physiologischer NaCl-Lösung, ferner einem Scintillogramm nach einer derartigen Spülung und schließlich mit einem Scintillogramm nachdem alle Schleimhaut aus beiden Nasenweiten herauspräpariert worden war.

Diese Methodik bringt den Nachweis, daß bestimmte Mengen der angebotenen markierten Substanz zum Zeitpunkt der Messung in der Nasenschleimhaut abgelagert sind, daß jedoch auch noch freies Albumin auf der Schleimhaut-Oberfläche befindet und nicht resorbiert wurde.

4. Die Messung der Aktivität in den Ausscheidungen war im Rahmen der verwendeten Substanzen und Nuklide nur beim Hippuran interessant, da dieses Modell für ein Dipeptid bekanntlich stark harngängig ist. Die Aktivität im Harn war dementsprechend bei Hippuran-Versuchen sehr hoch. Wir verzichten hier auf die Wiedergabe der Meßwerte.

Abb. 5 zeigt nun eine Gegenüberstellung der lokal gemessenen Aktivitäten über der Nasenschleimhaut und der gleichzeitig gemessenen Aktivitäten im



## Zusammenstellung der untersuchten Substanzen

Aufgetragene Substanz	Größe der Substanz
$J^{125}$	—
Hippuran- $J^{125}$	~400 Molekulargewicht
Lysozym- $J^{125}$	~14 000 Molekulargewicht 50 70 Å Teilchengröße
RISA ( $J^{125}$ markiertes Human-Albumin)	~69 000 Molekulargewicht 150 38 Å Teilchengröße
Kolloidale Lösung von Au <sup>198</sup>	109 145 Å Teilchengröße

Abb. 2 Zusammenstellung der untersuchten Substanzen

durch *autoradiographische* Untersuchungen der Schleimhaut während und nach der Resorption. Unsere diesbezüglich laufenden Versuche sind jedoch noch nicht so weit ausgereift, als daß sie hier zur Diskussion gestellt werden können.

Das oben erwähnte Prinzip der Kombination verschiedener Direktmessungen wurde von uns in den letzten Jahren ausgebaut und an einer Reihe von Substanzen mit unterschiedlichem Molekulargewicht angewendet. Von einigen der dabei erhaltenen Ergebnissen soll jetzt die Rede sein.

Versuchstier war aus praktischen Gründen das Kaninchen.

Die Abb. 2 gibt eine Übersicht über die bisher geprüften Substanzen. Als Nachweisverfahren wurden die folgenden in der Isotopentechnik erprobten Meßverfahren eingesetzt:

1. Die Aktivitätsmessung über der resorbierenden Schleimhaut. Areal. Sie erfolgte während einer durchschnittlichen Versuchsdauer von 90 min mit einem in 3 cm Abstand von der Nasenaußenfläche angebrachten blei abgeschirmten Scintillations Detektor, nachdem ein definiertes Volumen der zu prüfenden Substanz (10 mm<sup>3</sup>) auf die vordere Septumhälfte — bei seitlich liegendem Kopf — aufgebracht worden war. Da wegen des Selbstreinigungsapparates der Mukosa (Cilien + Schleimfilm) die Gefahr besteht, daß evtl. Testsubstanz über die Choana abtransportiert und damit an anderer Stelle des Atem- oder Verdauungstraktes resorbiert werden konnte, wurde der Nasenrachen der Versuchstiere mit Tamponade dicht abgestopft und bei Versuchsende die Aktivität auch in dieser Tamponade separat gemessen. Sie betrug dort maximal 2–5 % der aufgetragenen Gesamtaktivität. Abb. 3 zeigt einige Ergebnisse bei verschiedenen zur Resorption angebotenen markierten Substanzen.

2. Zur Messung der resorbierten markierten Substanzmengen wurde deren Konzentration im Blut bestimmt. Über die V. Jugularis der dem Auftragsort gegenüberliegenden Körperseite wurde ein Herzkatheter geschoben und in bestimmten zeitlichen Abständen je 5 ml Blut entnommen. Im daraus

sprechend besserer Resorptions-Erwartung, unsere Meßergebnisse verfälscht haben könnten.

Zur Klärung des Sachverhaltes hat es sich an, im Jeweils bei den Human-Albumin-Versuchen gewonnenen Serum eine Trennung zwischen eiweißgebundenem  $J^{131}$  und freien  $J^{131}$  Ionen vorzunehmen. Wir verwendeten vier verschiedene Methoden, von welchen sich aber nur zwei bewährten.

Nur von diesen beiden letzteren Nachweis-Methoden soll kurz die Rede sein.

(a) Die Filtration des Serums unter Druck durch ein Eiweiß-Membranfilter (Sartorius)

**Methodik.** Das Filtermaterial dieser Membranfilter besteht aus Zellulose-Derivaten und anderen Polymer Stoffen und zeichnet sich grundlegend durch seine strukturelle Gleichmäßigkeit vor anderen Filter Medien aus. Wir verwendeten das Membranfilter SM 12136, für Eiweiß-Stoffe bis zu einem Molekulargewicht von 10 000. Die Filtration von 20 cm Serum erfolgte in einem Druckfiltrationsgerät (Sartorius MD 50) unter gleichmäßigem Überdruck von 10 atü. Die Aktivität von Filtrat und Rückstand auf dem Filter wurden getrennt bestimmt.

Bei diesem Verfahren zeigt sich, daß im Blut Serum in dieser Versuchsserie nur 20% echtes mit  $J^{131}$  markiertes Human-Albumin nachzuweisen ist, während 80% der Serum Aktivität freiem  $J^{131}$  entl. auch markierten Eiweiß-Fragmenten mit einem Molekulargewicht von weniger als 3 000 entsprechen.

(b) Bei dem zweiten Verfahren, der Sephadex-Gel Filtration, wird das zu prüfende Serum auf eine 50 cm hohe Sephadex Säule gefüllt. Sephadex ist ein modifiziertes Dextran, ein sog. Molekular Sieb.

Die Polysaccharidketten des Sephadex sind zu einem dreidimensionalen Netzwerk verknüpft. Das stark hydrophile Gel hält Ionen zurück und läßt z. B. Eiweiß-Stoffe passieren. Wir verwendeten Sephadex G-75 normal, welches einen Fraktionierungsbereich für Peptide und Proteine zwischen 3 000 und 70 000 besitzt.

Das Filtrat wurde im Fraktionensammler aufgefangen und seine Aktivität gemessen. Abb. 6 zeigt das Ergebnis dieser Versuchsserie. Während eine — nicht dem Resorptionsvorgang unterworfen — RISA Lösung (Kontrolle) rasch mit ihrer eiweißgebundenen  $J^{131}$  Fraktion das Gel passiert, ist nur eine verhältnismäßig kleine Fraktion von freiem  $J^{131}$  nachweisbar — Fast umgekehrt verhält es sich bei dem Serum, das bei entsprechenden Resorptionsversuchen mit RISA aus dem Blut der Versuchstiere gewonnen wird. Hier ist der Anteil des an Eiweiß gebundenen  $J^{131}$  nur etwa 20%. Ihm stehen etwa 80% freies  $J^{131}$  gegenüber. Dies entspricht dem Ergebnis, das über ein Eiweiß-Membranfilter gemessen wurde.

Hieraus ergibt sich

1. Aktivitätsmessungen im Blute können bei Resorptionsversuchen irreführend sein. Es ist jeweils zu prüfen, ob der ganze zur Resorption angebo-



Abb. 5

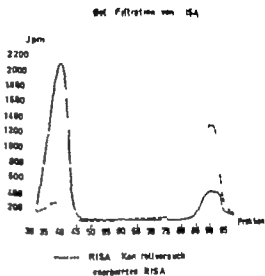


Abb. 6.

Abb. 5. Gegenüberstellung der Aktivitätskurven: oben bei Direktmessung über der Nase und unten bei Messung im Blut

Abb. 6. Sephadex Filtrat mit Human-Albumin (RISA) Resorption und ——— markiert in Human Albumin, welches über die Schleimhaut resorbiert worden war. Man erkennt beim „nativen“ Albumin den hohen Anteil an komplett im Albumin-Komplex (Fraktion 30–45) und den geringen Anteil an freiem Nuklid (Fraktion 80–95) Umgekehrtes Verhalten bei „resorbiertem Albumin“

Blut Auf einen einfachen Nenner gebracht, zeigt sich daß  $J^{131}$  sehr rasch, kolloidales Gold jedoch überhaupt nicht meßbar im Blut aufgenommen wird. Zwischen diesen beiden Extremen liegt abgestuft das Verhalten der anderen Substanzen mit unterschiedlichem Molekulargewicht, wobei die Resorptionsrate/Zelleinheit umgekehrt proportional zum Molekulargewicht zu sein scheint. — Dieses Ergebnis entsprach unseren theoretischen Erwartungen. Bei kritischer Betrachtung schien uns aber die Aktivitätskurve im Blut für Human Albumin (RISA) mit einem Molekulargewicht von etwa 69 000 suspekt. Nach dieser Kurve müßte es nämlich trotz des hohen Molekularvolumens anfangs zu einem verhältnismäßig sehr raschen Übertritt ins Blut gekommen sein. Es entstand der Verdacht daß die mit  $J^{131}$  markierte Testsubstanz entgegen der allgemeinen Annahme vielleicht nicht stabil inhomogen und/oder verunreinigt sein konnte, sodaß möglicherweise markierte Bruchstücke des Gesamt-Eiweiß-Komplexes oder sogar das Nuklid selbst Partikel also mit sehr viel kleinerem Molekulargewicht und damit

sprechend besserer Resorptions-Erwartung, unsere Meßergebnisse verfeinert haben könnten

Zur Klärung des Sachverhaltes hat es sich an, im Jeweils bei den Human Albumin Versuchen gewonnenen Serum eine Trennung zwischen eiweißgebundenem  $J^{125}$  und freien  $J^{125}$ -Ionen vorzunehmen. Wir verwendeten vier verschiedene Methoden, von welchen sich aber nur zwei bewährten.

Von diesen beiden letzteren Nachweis-Methoden soll kurz die Rede sein

(a) Die Filtration des Serums unter Druck durch ein Eiweiß-Membranfilter (Sartorius)

**Methodik** Das Filtermaterial dieser Membranfilter besteht aus Zellulose-Derivaten und anderen Polymer Stoffen und zeichnet sich grundlegend durch seine strukturelle Gleichmäßigkeit vor anderen Filter Medien aus. Wir verwendeten das Membranfilter SM 12136, für Eiweiß-Stoffe bis zu einem Molekulargewicht von 10 000 Die Filtration von 20 cm<sup>3</sup> Serum erfolgte in einem Druckfiltrationsgerät (Sartorius MD 80) unter gleichmäßigem Überdruck von 10 atü Die Aktivität von Filtrat und Rückstand auf dem Filter wurden getrennt bestimmt.

Bei diesem Verfahren zeigt sich, daß im Blut-Serum in dieser Versuchsserie nur 20% echtes mit  $J^{125}$  markiertes Human Albumin nachzuweisen ist, während 80% der Serum-Aktivität freiem  $J^{125}$  evtl auch markierten Eiweiß-Fragmenten mit einem Molekulargewicht von weniger als 2 000 entsprechen.

(b) Bei dem zweiten Verfahren, der Sephadex-Gel Filtration, wird das zu prüfende Serum auf eine 50 cm hohe Sephadex Säule gefüllt. Sephadex ist ein modifiziertes Dextran, ein sog Molekular Sieb.

Die Polysaccharidketten des Sephadex sind zu einem dreidimensionalen Netzwerk verknüpft. Das stark hydrophile Gel hält Ionen zurück und läßt z. B. Eiweiß-Stoffe passieren. Wir verwendeten Sephadex G-75 normal, welches einen Fraktionierungsbereich für Peptide und Proteine zwischen 3 000 und 70 000 besitzt

Das Filtrat wurde im Fraktionsammler aufgefangen und seine Aktivität gemessen. Abb. 6 zeigt das Ergebnis dieser Versuchsserie. Während eine — nicht dem Resorptionsvorgang unterworfenen — RISA-Lösung (Kontrolle) rasch mit ihrer eiweißgebundenen  $J^{125}$  Fraktion das Gel passiert, ist nur eine verhältnismäßig kleine Fraktion von freiem  $J^{125}$  nachweisbar — Fast umgekehrt verhält es sich bei dem Serum, das bei entsprechenden Resorptionsversuchen mit RISA aus dem Blut der Versuchstiere gewonnen wurde. Hier ist der Anteil des an Eiweiß gebundenen  $J^{125}$  nur etwa 20%. Ihm stehen etwa 80% freies  $J^{125}$  gegenüber. Dies entspricht dem Ergebnis, das über ein Eiweiß-Membranfilter gemessen wurde.

Hieraus ergibt sich

1. Aktivitätsmessungen im Blute können bei Resorptionsversuchen irreführend sein. Es ist Jeweils zu prüfen, ob der ganze zur Resorption angebo-

lene markierte Komplex im Blut messend nachgewiesen wird oder nur markierte Bruchstücke oder gar isolierte Nuklide

2 Es läßt sich nachweisen daß Human Albumin von der Nasenschleimhaut aufgenommen wird Seine Menge entspricht jedoch nicht der im Blut gemessenen Aktivitätskurve In der Gesamtbilanz ist von einem definierten auf die Mukosa aufgetragenem Human Albumin nach 90 min Versuchsdauer nur etwa 1% Aktivität im Blut nachweisbar Von diesem 1% ist jedoch nur etwa 1/5 erweißgebundenes  $J^{125}$  also Human Albumin 4/5 der Aktivität sind entweder durch freies  $J^{125}$  oder durch bis jetzt nicht näher definierbare markierte Eiweiß-Bruchstücke mit einem Molekulargewicht von weniger als 3 000 verursacht.

3 Es ist die Frage noch völlig offen, ob diese im Blut nachweisbare Differenzierung in echtes markiertes Erweiß und Eiweiß-Bruchstücke bzw. freies Nuklid lediglich durch eine Filterwirkung der Schleimhaut bei der Resorption zustande kommt ob sie bereits die Folge einer spaltenden Enzymtätigkeit in der Mukosa — also nach stattgehabter Resorption — darstellt oder ob etwa die Gefäßwände in der Schleimhaut für eine filternde Trennung verantwortlich sind

4 In bestimmten Grenzen ist die Resorptionsrate in der Zeiteinheit anscheinend indirekt proportional zum Molekulargewicht der zu resorbieren den Substanz.

o Auf eine sehr interessante Feststellung ist noch hinzuweisen Die Partikelgröße von kolloidalem Gold ist rund 150 Å im Durchmesser die von Human Albumin  $150 \times 38$  Å Die Partikel beider Substanzen liegen etwa in der gleichen Größenordnung Nach unseren bisherigen orientierenden Messungen werden beide Substanzen in geringem Umfang in die Schleimhaut aufgenommen Während jedoch Albumin dann auch im Blut nachweisbar wird ist dies bei kolloidalem Gold nicht der Fall Hier muß also außer der Partikelgröße noch ein anderer wesentlicher Faktor die Resorptionsrate mit bestimmen

Aus diesen Feststellungen ergeben sich weitere Fragen, so z. B.

(a) Human Albumin (Teilchengröße  $150 \times 38$  Å) wird von der Mukosa resorbiert und ist im Blut nachweisbar kolloidales Gold (Teilchengröße  $\sim 150$  Å) erscheint nicht im Blut Wo liegt die kritische Teilchen-Größe für die Aufnahme in die Nasen Mukosa? Welches ist die kritische Größe für Partikel, die — von der Mukosa resorbiert — noch die Wand der Schleimhaut Gefäße passieren können? Welche Mechanismen oder Prinzipien in der Schleimhaut sind für diese Selektion gleichgroßer Partikel in blutgängige und nicht blutgängige Substanzen verantwortlich?

(b) Es ist nachgewiesen (Chevance, 1957 H. H. Naumann, 1958 Strömme 1952) daß von sensibilisierter Schleimhaut noch sehr viel größere Partikel als kolloidales Gold aufgenommen werden können, z. B. Pollen (Roggenpollen z. B.  $30 \mu$ ) Über welche Mechanismen ist dies möglich? Handelt es

sich hierbei noch um Resorption oder etwa um eine aktive Leistung der eindringenden Partikel

(c) Wo speichert die Mukosa resorbierte Substanzen und was geschieht weiter mit ihnen?

(d) Wie verhält sich der Resorptionsvorgang, wenn entweder die physikalischen oder chemischen Eigenschaften der zu resorbierenden Stoffe abgewandelt werden oder andererseits der Zustand der Mukosa selbst verändert wird?

### RÉSUMÉ

Plusieurs méthodes pour établir la résorption de la muqueuse nasale et des difficultés considérables quant à l'identification de substance radioactives dans le sang resorbées par la muqueuse. La dimension de la radioactivité trouvée dans le sang et dans le crum correspond pas forcément à la substance radioactiv appliquée à la muqueuse nasale. Par conséquent nous tenons à la comparaison des substances trouvées dans le sang avec les substances appliquées à la muqueuse nasale suivant des méthodes de recherche qui permettent cette comparaison.

### SUMMARY

Several methods for measuring the resorption capacity of the nasal mucous membrane are critically discussed. The experimental results communicated in this paper have been obtained by the  $J^{131}$  and  $J^{125}$  traced substances (hippuran, lysozyme human albumin) as well as by colloidal  $Au^{198}$ . Different ranges have been combined (a) directly computing (b) in the region of resorption (c) in the blood, (d) in the excretions, and (e) by scintiscanning. It could be proved the computed activities from the blood are of the same as those from the whole traced complex being resorbed (i.e. human albumin). We could demonstrate by our experiment on albumin resorption that the total activity computed from the blood had been split up into albumin-complex and free iodine or fragmented albumin. Possible causes for this phenomenon are discussed.

### LITERATUR

- BOLIN, O. 1965 Die Schleimhaut resorptionsfähigkeit etc. *Z. Laryng Rhinol Otol* 45 329
- BROCH, G., H. und FERRIS, L. 1968 Über die Aufnahme markierter Glukose an Schleimhäuten. *Verh. Dtsch. Med. Ges. Mitt. Jahrb. Arch. Oh. Nas. K. H. H. pfeil* 191 im Druck.
- CHEN, G. L. G. 1945 Contribution à l'étude expérimentale de l'écoulement nasal. *J. Otolaryng.* (P.) 54 26
- KLING, H. H. 1939 Itraalbeobachtungen der Nasenschleimhaut. *Arch. Oh. Nas. K. H. H. pfeil* 11 173 177
- 1938 Die Aufnahme körperfremder Stoffe in die Nasenschleimhaut. *Fortchr. HNO-H. K. H. H. (Basel)* 3, 167
- KLING, H. H. und MÜLLER, M. 1948 Resorptionsmessungen der Nasenschleimhaut des Kaninchens. *Arch. Oh. Nas. K. H. H. pfeil* 191 im Druck.
- STÄNDLER, O. 1932 Studies on the histopathology of polyps. *Act. Otolaryng.* (Stockh.) Suppl. 10
- TOMMERS, J. H. DE, R. V. CHIN, H. J. et al. 1933 Absorption from the nasal mucous membrane. *Ann. Otol.* 42 337

HNO-Klinik der Freien  
Universität Berlin,  
Berlin, Deutschland

## DISCUSSION

*T Palva* We know from some English studies on animals that various substances instilled into the nose are taken up with the lymph vessels and they enter the circulation in this way. Has Mr Naumann made any studies in this respect and what is his opinion of the part played by the lymph vessels and the capillaries in the absorption?

My second question has to do with the effect of parasympathetic and sympathetic stimulation in the nasal mucosa. Since the former causes a swelling of the mucosa and the latter a constriction of the blood vessels and mucosa, the effect on absorption could also be very much different. Is it possible that the substances introduced into the nose by Mr Naumann could as such, also cause damage in the normal basic nasal mucosal balance?

*G F Greiner* Je voudrais avoir l'opinion de Mr Naumann sur la résorption de la molécule S par la muqueuse nasale. Question qui se pose pour l'activité des Eaux Thermales.

*H Engström* I am especially interested in the behaviour of his radioactive gold particles. In studies carried out by me and my colleagues, it has been found that different colloidal silver chemicals are taken up in a very different way although they should be very similar in molecular size. From the literature we know that colloidal silver for some reason electrical chemical or other has a pronounced affinity for the olfactory sensory cells. There seems to be a difference between the uptake of different chemicals between supporting and sensory cells. Have you in the olfactory region of the nasal mucosa, seen any difference in uptake between supporting cells and olfactory cells? Have you seen a very high uptake in sensory cells or neural elements?

*H H Naumann* (Antwort) an Hr *Palva* Die angegebenen Substanzen haben wahrscheinlich keinen Effekt auf das vegetative Nervensystem. Es ist zu erwarten dass die Resorptionsrate sich ändert wenn die Mukosa unter sympathischer oder auch parasympathischer Stimulation steht. Unsere diesbezügliche Experimentreihe ist noch nicht abgeschlossen. Wir sind z. Z. dabei, auch die Aktivität in der Lymphe der Nasenschleimhaut zu messen. Dies macht noch gewisse methodische Schwierigkeiten. Allem Anschein nach sind Lymphkapillaren für Partikel grosser Molekulargewichte besser durchgängig als entsprechende Blutkapillaren.

An Hr *Greiner* Kolloidaler Schwefel wurde vor uns noch nicht in die Versuche einbezogen.

An Hr *Engström* Differenzierte Untersuchungen darüber ob sensorisches oder respiratorisches Epithel oder Stützellen unterschiedlich beim Resorptionsvorgang mitwirken, haben wir nicht angestellt. Vermutlich sind für die Resorption nicht nur die Partikelgrösse, sondern auch Faktoren wie die elektrische Ladung, der Isoelektrische Punkt etc. wichtig so dass Unterschiede der verschiedenen Epithelarten bei der Teilnahme am Resorptionsvorgang denkbar sind.

## EXPERIMENTAL TRANSFER OF ALLERGIC INFORMATION IN LABORATORY ANIMALS

S. PODVINEC, R. ANDRIĆ, V. SAVIĆ, B. MITROVIĆ and M. ANOŠIĆ

*From the Clinic of Otolaryngology Medical Faculty University of Belograd and  
the Clinic of Surgery Faculty of Veterinary Medicine University of Belograd  
Belograd Yugoslavia*

In three series of experiments on guinea pigs the authors have transferred sensitization to ovalbumin from sensitized animal to normal animals by means of a temporary skin graft which was implanted after staying in the recipient for 7 days. In the same way desensitization could also be transferred as an immunological information to sensitized animals. The authors conclude that sensitization and desensitization are coded and memorized in cell elements and can be transferred to a homologous animal in the shape of a molecular graft which becomes incorporated into the genetic system of immunologically competent cells.

Interest in respiratory allergy is steadily increasing. It is not only centered around the purely allergic diseases like pollinosis or allergic rhinitis. It is not caused only by the fact that many diseases of the respiratory tract are the result of a combined action of allergy and infection, like subglottic laryngitis and asthma. It is more and more widely recognized that in most instances chronic infection of the air passages has allergy as its basis and can be cured successfully only if both nosologic factors are treated. Urbanization and industrialization in contemporary life are responsible for an increase in the occurrence of occupational allergy which is a problem in many fields of industry particularly in chemical factories. Very severe manifestations of allergy to drugs can appear in the respiratory organs, such as oedema of the larynx, particularly following administration of antibiotics and certain vitamins. An ever-increasing number of patients are coming to the department of allergy in our clinic who belong to one of these four groups. The greatest number belong to the group of chronic infection on which the allergic terrain can be proven in 70-80 per cent of the cases.

The theoretical studies of the problems of allergy are becoming more and more challenging since the transplantation of organs to human beings has become a feature of the most spectacular progress in medicine. The immunological processes of rejection or tolerance to a graft are touching the fundamental questions of allergic reactions.

In the course of our experimental studies we have executed three series of experiment on guinea pigs. In all instances the method to test the



## DISCUSSION

*T Palva* We know from some English studies on animals that various substances instilled into the nose are taken up with the lymph vessels and they enter the circulation in this way. Has Mr Naumann made any studies in this respect and what is his opinion of the part played by the lymph vessels and the capillaries in the absorption?

My second question has to do with the effect of parasympathetic and sympathetic stimulation in the nasal mucosa. Since the former causes a swelling of the mucosa and the latter a constriction of the blood vessels and mucosa, the effect on absorption could also be very much different. Is it possible that the substances introduced into the nose by Mr Naumann could, as such, also cause damage in the normal basic nasal mucosal balance?

*G F Greiner* Je voudrais avoir l'opinion de Mr Naumann sur la résorption de la molécule S par la muqueuse nasale. Question qui se pose pour l'activité des Eaux Thermales.

*H Engström* I am especially interested in the behaviour of his radioactive gold particles. In studies carried out by me and my colleagues, it has been found that different colloidal silver chemicals are taken up in a very different way although they should be very similar in molecular size. From the literature we know that colloidal silver for some reason electrical chemical or other has a pronounced affinity for the olfactory sensory cells. There seems to be a difference between the uptake of different chemicals between supporting and sensory cells. Have you, in the olfactory region of the nasal mucosa, seen any difference in uptake between supporting cells and olfactory cells? Have you seen a very high uptake in sensory cells or neural elements?

*H H Naumann* (Antwort) an Hr Palva Die angegebenen Substanzen haben wahrscheinlich keinen Effekt auf das vegetative Nervensystem. Es ist zu erwarten dass die Resorptionsrate sich ändert, wenn die Mukosa unter sympathischer oder auch parasympathischer Stimulation steht. Unsere diesbezügliche Experimentreihe ist noch nicht abgeschlossen. Wir sind z. Z. dabei, auch die Aktivität in der Lymphe der Nasenschleimhaut zu messen. Dies macht noch gewisse methodische Schwierigkeiten. Allem Anschein nach sind Lymphkapillaren für Partikel grosser Molekulargewichte besser durchgängig als entsprechende Blutkapillaren.

An Hr Greiner Kolloidaler Schwefel wurde vor uns noch nicht in die Versuche einbezogen.

An Hr Engström Differenzierte Untersuchungen darüber ob sensorisches oder respiratorisches Epithel oder Stützellen unterschiedlich beim Resorptionsvorgang mitwirken haben wir nicht angestellt. Vermutlich sind für die Resorption nicht nur die Partikelgrösse, sondern auch Faktoren wie die elektrische Ladung, der isoelektrische Punkt etc. wichtig, so dass Unterschiede der verschiedenen Epithelarten bei der Teilnahme am Resorptionsvorgang denkbar sind.

## EXPERIMENTAL TRANSFER OF ALLERGIC INFORMATION IN LABORATORY ANIMALS

S. PODVINEC, R. ANDRIĆ, V. SAVIC, K. MITROVIĆ and M. ANOŠIĆ

*From the Clinic of Otolaryngology Medical Faculty University of Beograd and  
the Clinic of Surgery Faculty of Veterinary Medicine University of Beograd  
Beograd, Yugoslavia*

In three series of experiment on guinea pigs the authors have transferred sensitization (albumin from sensitized animals to normal animals by means of temporary skin graft which was explanted after staying in the recipient for 7 days. In the same way desensitization could also be transferred as an immunological information to sensitized animals. The authors conclude that sensitization and desensitization are coded and memorized in cell element and can be transferred to homologous animal in the shape of a molecular graft which becomes incorporated into the genetic system of immunologically competent cells.

Interest in respiratory allergy is steadily increasing. It is not only centered around the purely allergic diseases like pollinosis or allergic rhinitis. It is not caused only by the fact that many diseases of the respiratory tract are the result of a combined action of allergy and infection, like subglottic laryngitis and asthma. It is more and more widely recognized that in most instances chronic infection of the air passages has allergy as its basis and can be cured successfully only if both nosologic factors are treated. Urbanization and industrialization in contemporary life are responsible for an increase in the occurrence of occupational allergy which is a problem in many fields of industry particularly in chemical factories. Very severe manifestations of allergy to drugs can appear in the respiratory organs, such as oedema of the larynx, particularly following administration of antibiotics and certain vitamins. An ever-increasing number of patients are coming to the department of allergy in our clinic who belong to one of these four groups. The greatest number belong to the group of chronic infection on which the allergic terrain can be proven in 70-80 per cent of the cases.

The theoretical studies of the problems of allergy are becoming more and more challenging since the transplantation of organs to human beings has become a feature of the most spectacular progress in medicine. The immunological processes of rejection or tolerance to a graft are touching the fundamental questions of allergic reactions.

In the course of our experimental studies we have executed three series of experiments on guinea pigs. In all instances the method to test the

presence of allergic systematic sensitization was performed according to the experience of Mojović & Škerović (1964) on the surviving heart of the guinea pig. The animal is sacrificed and the heart is rapidly excised and fixed to the end of a tube in Langendorff's apparatus, where it can be kept beating for 20 to 30 minutes owing to a perfusion under constant temperature pressure and oxygenation. The autonomous contractions of the heart can easily be registered mechanically as we did in the beginning, or with the help of a mechanotransducer as we did more recently.

When the heart of an animal is examined in this way 2 weeks after intraperitoneal sensitization with ovalbumin, it will after having established regular contractions, change instantly its frequency and amplitude when a few drops of the specific antigen are added to the perfusing solution (Fig 1). After some 10 or more minutes the disturbance of the action of the heart becomes more intense and develops into an atrio-ventricular bloc (Fig 2). After some lapse of time the heart can recover again its former regular beat which shows that it was the allergic reaction, and not the trauma of the experiment, which brought about the change registered in the graph.

In the first series of experiments we started with a number of guinea pigs sensitized to ovalbumin. After having examined positive and negative control animals we desensitized our group with Subtivaccine. This drug, won and produced on the basis of the autolysis of non pathogenic bacteria, *B. Subtilis* and *B. megatherium* when given to our patients has in clinical practice shown great potency in the hypsensitizing treatment of respiratory allergy. As in clinical treatment Subtivaccine was employed on the surface of the skin which was scarified on the abdomen of the animals twice a week during 6 weeks. The animals were sacrificed after the end of the treatment. Their surviving heart was examined by the method described. When the specific antigen was added to the perfusing solution there was no perceptible reaction, the heart beat was unperturbed (Fig 3).

In the second series of experiments we tried to effect the transfer of allergic information from a sensitized to a normal animal. A piece of skin was taken from the abdomen of a sensitized donor animal and transplanted to the abdomen of a normal recipient animal. The full thickness graft was left in place only for 7 days and then it was surgically removed. Two weeks later both the donor and the recipient animal were sacrificed and their hearts examined in the Langendorff apparatus. It was found that both the donor as well as the recipient animal reacted promptly to contact with allergen. In one group of animals ovalbumin was used as antigen. The graph obtained from the recipient animal (Fig 4) was no less positive than the one from the donor animal the one that had been sensitized. The heart soon went into dissociation of its contractions (Fig 5). Even more pronounced and constant was the transfer of the information of sensitization when the donor was sensitized with horse serum. The recipient showed intensive depression of the contraction (Fig 6) and atrio-ventricular bloc.

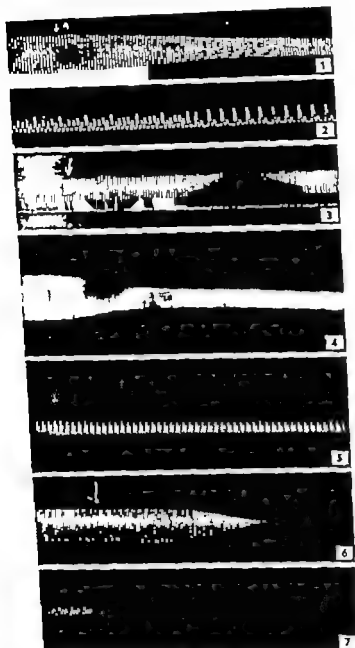


FIG. 1

(Fig. 7) In this second group of animals all five were equally positive, while in the ovalbumin group of five animals were positive.

In a third series of experiments we tried to effect the transfer of anti-allergic information to sensitized animals. Starting with two groups of guinea pigs, one sensitized with ovalbumin and the other first sensitized with ovalbumin and then desensitized with Subtilvacine, full thickness

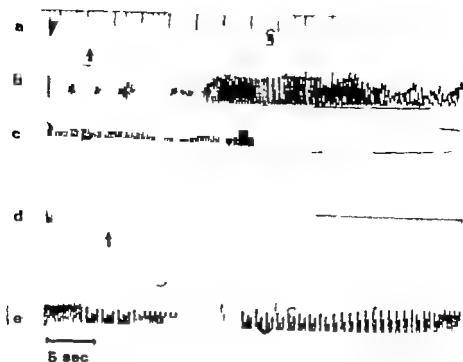


FIG. 8

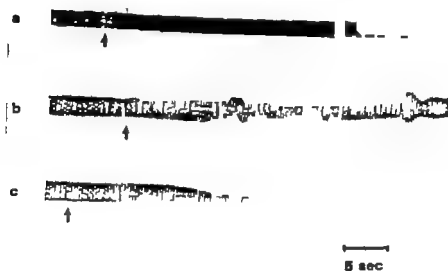


FIG. 9

skin grafts were taken from desensitized donors and temporarily implanted into sensitized recipients. In this series of experiments we repeated the observations we made in our first and second series (Fig 8). Our positive controls i.e. the sensitized animals, showed prompt depression of the heart beat on contact with ovalbumin (A) atrio-ventricular bloc some minutes later (B) and still later recovery (C) which shows the heart was not damaged in the experiment. The desensitized animals showed no reaction on contact with the antigen (D). Some desensitized animals used as controls showed a slight positive response to the allergen (E) (two animals in eight in this experiment).

In the group of sensitized recipients which had been carrying the skin (from desensitized donors for 7 days and which were sacrificed 2 weeks after the explantation of the skin one half of the total number (four in eight) manifested complete desensitization (Fig. 9 A). Two animals of the recipient group had a high degree of desensitization (B) and only two were slightly positive to the specific antigen (C) not reaching at all the strong positive reaction of the positive controls. Of six positive controls there were three strongly positive, two positive, and one uncertain. In a control group of eight animals desensitized directly with Sublinvaccine two did not respond to contact with the allergen, four responded slightly and two were uncertain.

### CONCLUSIONS

1. Allergic sensitization in guinea pigs is a pattern of specific immunological reaction which is coded in cellular elements. This code of behaviour remains memorized in these cells and can be transferred to other homologous animals.

2. Desensitization and hyposensitization can be effected in sensitized animals by employing Sublinvaccine on the surface of the skin by the scarification method. Desensitization can also be coded and memorized in cell elements and can be transferred to other homologous animals.

3. The "transfer factor" first suggested by Chase (1945) then demonstrated by Lawrence (1955), Lawrence & Pappenheimer (1956) and Lawrence (1960) comes to evidence in our experiments. There is a possibility that the "transfer factor" is a molecular graft which becomes incorporated into the genetic system of the nuclei of immunologically competent cells and is carried over to subsequent generations of these cells, directing the pattern of the immunological behaviour of the individual.

### RÉSUMÉ

Dans trois séries d'expériences sur le cobaye les auteurs ont réussi à transmettre la sensibilisation. Ovalbumine de l'animal sensibilisé à l'animal normal comme un transfert d'information immunologique. Ils se sont servis d'une greffe cutanée dont l'animal sensibilisé était le donneur et l'animal normal le bôte. La greffe cutanée était explantée après 7 jours. On a trouvé qu'on peut transmettre la désensibilisation dans la même façon par une greffe à un animal homologue. Les auteurs pensent que la sensibilisation et la désensibilisation sont fixées dans la mémoire des éléments cellulaires et que ce programme peut être transmis dans la forme d'un greffe moléculaire à un animal homologue. Elle peut être incorporée dans le système génétique des cellules d'ordre immunologique.

### ZUSAMMENFASSUNG

In drei Serien von Experimenten an Meerschweinchen ist es den Autoren gelungen eine Sensibilisierung auf Ovalbumin von den sensibilisierten Tieren auf normale Tiere zu übertragen indem sie dazu ein Hauttransplantat benutzten, das

nur sieben Tage im Empfänger verweilte. Auf dieselbe Weise konnte auch eine Desensibilisierung als immunologische Information auf sensibilisierte Tiere übertragen werden. Die Autoren nehmen an, dass Sensibilisierung wie auch Desensibilisierung als Programm in Zellelementen aufbewahrt werden und auf homologe Tiere in Form eines molekulären Transplantates übertragen werden kann. Dieses kann in das genetische System immunologisch aktiver Zellen eingebaut werden.

## REFERENCES

- LAWRENCE A. S. RAPAPORT P. T. COVARR, J. M. and TILLEY W. S., 1962 Homograft sensitivity in human beings *Transplantation* (Ciba Symposium) J. & A. Churchill, London.
- MOJČIĆ, M. and ŠERNOVIĆ D.: 1964, Ponašanj izloženog sren sensibiliziranih zamoraca u toku reaktivne antigenitne reakcije *Acta Med Jugosl.* 18: 9.
- PODVIŃEC, S. MOJČIĆ, M. MITROVIĆ, K. VUČIĆEVIĆ, D. and ANDRIJAŠEVIĆ, I.: 1966 Recherches expérimentales et cliniques sur la désensibilisation péférique dans la maladie allergique des voies respiratoires. *Ther Umsch.* 23: 432.

*Clinic of Otolaryngology Medical Faculty Clinic of Belgrade Belgrade Yugoslavia*

## DISCUSSION

J. E. Bordley: Have only immediate transplants been done? Has freezing of the graft with delayed transplant been attempted? Have animals been challenged after a longer period than 2 weeks following transplant? What evidence of rejection has there been of the sensitized grafts and of the desensitized grafts? Has white blood cell typing been carried out between donor and recipient?

H. H. Naumann: Wie wurde in der Versuchsserie der Grad der "Sensibilisierung" oder "Desensibilisierung" bestimmt und definiert? Wurde der Antikörper Titer im Blut festgestellt? Wurden sonstige immunologische Bestimmungen durchgeführt?

S. PodviŃec (Reply) to Mr. Bordley: We have used only immediate grafting which has seemed most appropriate for this work. We have never waited more than a week to explant the graft. By doing so we have not experienced the rejection of the graft though under the microscope the explanted skin has shown signs of reactions of intolerance like hyperaemia, granulation tissue and defective staining. The animals used in the experiments were random samples of animals from different families.

To Mr. Naumann: Wir haben es nicht als notwendig gefunden den Grad der Sensibilisierung oder der Desensibilisierung durch humorale Messungen zu bestimmen, obwohl das nicht ohne Interesse wäre. Wir hielten bisher an der einen Testmethode am überlebenden Herzen fest, weil sich diese Methode als sehr empfindlich gezeigt hat und weil sie über eine längere Beobachtungzeit (bis zu 30 Minuten) ausgedehnt werden kann und sich gut registrieren lässt.

## RECURRENZ KURZSCHLUSS-ANASTOMOSE ZUR RÜCKGEWINNUNG DER VERLORENEN STIMMLIPPENMOTILITÄT

Kurzbericht

A. MIEHLKE

*Aus der Universitäts-Hals-Nasen-Ohrenklinik Göttingen, Deutschland*

Experimenten an 10 Hunden wurde das Ziel gesetzt, an dem medialen Segment des Vagusstammes den in Höhe des Kehlkopfes bereits präformierten Recurrenzast einen Teil abzuspalten und diesen mit dem Kehlkopf nahen Recurrenzstumpf zu anastomosieren. Nach 7 bis 10 Monaten wurde der Erfolg der Reinnervation im Bereich der Kehlkopfmuskulatur morphologisch überprüft.

Eine Arbeitsgruppe um Miehke (Dal Ri, Schmidt, Küsel, Haubrich und Schäfers) untersuchten im Tierexperiment die Frage, ob eine Reinnervation der Stimmlippen nach einer Vagus-Recurrenzplastik zu erzielen ist. Dieses Problem ist von praktisch-klinischem Interesse, weil in ca. 0,5 (Lahey & Hoover 1938) bis 9,4% (Blomstedt & Rydmark) nach Strumaoperationen eine latrogene Läsion der Recurrentes stattfindet und auch in neuerer Zeit durch schwere Verkehrsunfälle immer häufiger Verletzungen der Recurrenten eintreten.

Die Wiederherstellung der normalen Funktion des N. recurrens wäre nach Operations- und Unfallverletzungen natürlich am einfachsten durch die primäre Nervennaht, wie sie zuerst Sierlin (1916), Lahey & Hoover (1938) angaben, bei breiteren Defekten durch die Autonerventransplantation Miehke (1967) zu erreichen.

Die Wiedervereinigung der Nervenstümpfe bei einem durchtrennten N. recurrens bereitet vielfach grosse Schwierigkeiten, da es sehr schwer sein kann, den Kehlkopf fernem Recurrenzastumpf im postoperativen Narbengewebe aufzufinden. Diese Schwierigkeit gilt nicht für den Kehlkopfnahen Stumpf des N. recurrens, da feste Markierungspunkte sicher zu ihm hinführen.

Zur Rückgewinnung der Stimmlippenmotilität ist die morphologische Wiederherstellung des peripheren Recurrenzneurons die Voraussetzung. Es wurde deshalb im Tierexperiment eine sogenannte Kurzschluss-Anastomose des N. recurrens gebildet. Es war das Ziel, an dem medialen Segment des Vagus den in Kehlkopfhöhe bereits präformierten Recurrenzastenteil (Abb. 1) unter dem Operationsmikroskop abzuspalten und diesen mit dem



nur sieben Tage im Empfänger verweilt. Auf dieselbe Weise konnte auch eine Desensibilisierung als immunologische Information auf sensibilisierte Tiere übertragen werden. Die Autoren nehmen an, dass Sensibilisierung wie auch Desensibilisierung als Programm in Zellelementen aufbewahrt werden und auf homologe Tiere in Form eines molekulären Transplantates übertragen werden kann. Dieses kann in das genetische System immunologisch aktiver Zellen eingebaut werden.

## REFERENCES

- LAWRENCE, A. S., RAPAPORT, F. T., CONVERSE, J. M. and TILLET, W. S., 1962 Homograft sensitivity in human being *Transplantation* (Ciba symposium) J. & A. Churchill, London.
- MOJČIĆ M. and ŠKROVIĆ D., 1964 Ponašanje i angrena senzibiliziranih pacijenata u toku reakcije na antigeni antitel *Acta Med Jugosl* 18: 9.
- PONTI, G., S. MOJČIĆ M., VITTORELLI, H., VUČIĆIĆ, B. and V. DJELKOVIĆ I. 1966 Recherches expérimentales et cliniques sur la désensibilisation et péritique dans la maladie allergique des voies respiratoire *The Larynx* 23: 422.

*Clinic of Otolaryngology and Medical Faculty University of Belgrade  
Belgrade Yugoslavia*

## DISCUSSION

J. F. Bordley: Have only immediate transplants been done? Has freezing of the graft with delayed transplant been attempted? Have animals been challenged after a longer period than 2 weeks following transplant? What evidence of rejection has there been of the sensitized grafts and of the desensitized grafts? Has white blood cell typing been carried out between donor and recipient?

H. H. Naumann: Wie wurde in der Versuchsreihe der Grad der Sensibilisierung oder „Desensibilisierung“ bestimmt und definiert? Wurde der Antikörper Titer im Blut festgestellt? Wurden sonstige immunologische Bestimmungen durchgeführt?

S. Podvišec (Reply) to Mr Bordley: We have used only immediate grafting which has seemed most appropriate for this work. We have never waited more than a week to explant the graft. By doing so we have not experienced the rejection of the graft though under the microscope the explanted skin has shown signs of reactions of intolerance like hyperaemia, granulation tissue and defective staining. The animals used in the experiments were random samples of animals from different families.

To Mr Naumann: Wir haben es nicht als notwendig gefunden den Grad der Sensibilisierung oder der Desensibilisierung durch humorale Messungen zu bestimmen, obwohl das nicht ohne Interesse wäre. Wir hielten bisher an der einen Testmethode am überlebenden Herzen fest, weil sich diese Methode als sehr empfindlich gezeigt hat und weil sie über eine längere Beobachtungszeit (bis zu 30 Minuten) ausgedehnt werden kann und sich gut registrieren lässt.

phologisch (Histologie Histochemie) sowie funktionell (direkte Laryngoskopie Elektromyographie, Zeitrafferfilm) objektiviert und im Vortrag anhand eines Filmes demonstriert.

Es konnte gezeigt werden, dass durch eine solche Vagus Recurrensplastik (Recurrent-Kurzschluss Anastomose) die echte Recurrensfunktion, wenn auch nicht vollständig, so doch in einem klinisch vielleicht ausreichenden Grade zurückzugewinnen ist.

Der interessierte Leser sei hinsichtlich aller Einzelheiten des experimentellen Vorgehens und der Auswertung der Ergebnisse auf die Originalarbeiten von Niehke und Mitarbeitern (*Arch Klin Exp Ohr Nas Kehlkopfheilk* 188 654-667 (1967) sowie 188 668-680 (1967) verwiesen.

### RÉSUMÉ

Chez 10 chiens on a détaché du segment médial de la racine pneumogastrique à la hauteur du larynx la partie contenant des fibres récurrentes déjà préformées et on les anastomosa sur le pédicule du récurrent à proximité du larynx. Après 7 à 10 mois l'aspect de la réinnervation fut objectivé dans le domaine morphologique et fonctionnel.

### SUMMARY

In experiments on dogs the part of the recurrent nerve already performed at larynx level and well visible in the vagus nerve was isolated and anastomized with the trunk of the recurrent laryngeal nerve near to the larynx. After a regeneration time of 7-10 months histological, histochemical, and electromyographic examination demonstrated that it is possible to achieve reinnervation of the laryngeal muscles by such form of neuroplastic procedure.

### LITERATUR

- BRUNNEN, J. 1951 Atmung und Stimme nach Larynxresektion oder Stimmrinne bei beidseitiger Postcriculärektomie. *Arch. Oh. Nas. Kehlkopfheilk* 150 225.  
 — 1954 Erfahrungen an Atmung und Stimme nach Stimmrinnektomie. *Med. Woch.* 81.  
 — 1956 Neue Ergebnisse über Bewegung gestörten Kehlkopf. *Arch. Oh. Nas. Kehlkopfheilk*, 163 1.  
 CATTAL, M. nach BRUNNEN (1954).  
 FRAENKEL-PETERSEN, A., 1961 Nicht rekurrierender Larynx inferior R. *Bras. Ci.* 22 317 ref. *Zbl. Hals. Nas. Ohrenheilk* 48 197 (1952 63).  
 LASKY, F. H., and HOOKER, W. B. 1938 Inj. dies. the recurrent laryngeal nerve in thyroid operations. *Ann. Surg.* 106, 545.  
 NIEHKE und HART 1967 cit. nach OVOO. *Die Anatomie und Physiologie der Kehlkopf- und Stimmorgane*. O. Cohnke, Berlin.  
 OVOO, R., 1916 cit. nach HOOKER. *H. J. Bruns Beitr. Klin. Chir.* 99 184.

Die U. Journalistik II V U  
 G. Göttingen 18 Göttingen /BRD  
 Deutschland

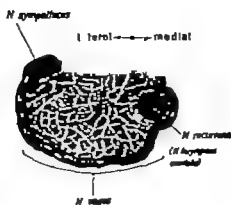


Abb. 1 Querschnitt durch den V. vagus beim Hunde in Höhe der Kehlkopfregion nach Chase und Ranson 1914.



A. a. 2. Vagus Recurrensplastik oder Recurrens-Kurzschlus-Anastomose nach Miehle

Kehlkopfnahen (stets sicher auffindbaren) Recurrenzsstumpf zu anastomosieren (Abb 2)

Die Berechtigung zu einem solchen Vorgehen ergibt sich durch die eigenartige Lageentwicklung des V. recurrens. Bekanntlich wird dieser aus seiner ursprünglichen Lage mit einem Kehlkopfnahen Abgang vom Vagusstamm beim Descensus cordis in die obere Thoraxapertur heruntergezogen. Diese ontogenetisch bedingte Verlagerung wird in einigen Beobachtungen nicht vollzogen. Cattell (zit. nach Berendes, 1956) fand bei 479 Strumektomien neunmal, daß der N. recurrens vom Vagus in Höhe des unteren Schilddrüsenknorpelrandes abging und ohne Umweg in die Kehlkopfmuskulatur eintrat und gleiche Beobachtungen liegen vor von Stedmann & Hart (1902), Lahey & Hoover (1938) sowie Frauzino Pereira (1951). Diese Rückbesinnung auf primitive Lagebeziehungen stellte das Modell für unsere Überlegungen dar.

Die Ergebnisse unserer Recurrensplastiken an 10 Hunden werden mor-

herstellung der peripheren Recurrensaneurons mit Hilfe der einfachen Naht hatte ich noch nicht Gelegenheit durchzuführen. Dagegen gelang es mir vor 2 Jahren, einen Defekt im Verlauf des  $\vee$  recurrens durch ein Autonerventransplantat zu überbrücken. Als Spendernerven verwendete ich den  $\vee$  auricularis magnus. Ich möchte Ihnen hier anhand einiger Diapositive das erfreuliche Ergebnis dieser Wiederherstellung des Recurrensverlaufes zeigen. Sie sehen, dass die Stimmlippenbeweglichkeit zu einem guten Teil und in ausreichendem Masse wiederhergestellt ist.

An Hr Engström, Hr Arslan, Hr Brunetti u d H Angell-James Ich danke für Ihre Bemerkungen, die zu weiterer Forschung in bestimmter Richtung anregen.

An H Jeschek Ich bin in der Lage, Ihnen einen Querschnitt durch den  $\vee$  vagus beim Menschen zu zeigen. Dieses Präparat wurde möglich, weil ich in einem Fall einer Neck Dissection bei Tumori vasion unter anderem in den  $\vee$  vagus, gezwungen war diesen unter der Schädelbasis zu resektieren. Deutlich kann man hier bereits die separierten und gebündelten Recurrensfasern im medialen Anteil des  $\vee$  vagus erkennen und ich konnte feststellen, dass die Separierung dieses Recurrensfaserbündels vom übrigen Vagus ohne jede Traumatisierung gelingt.

An Hr Jongkees Es handelt sich hier um Akut Experiment. Es ist wichtig — und ich danke für den Hinweis — dass in weiteren Versuchsreihen die Frage des zweckmäßigsten Zeitpunktes der Intervention geklärt werden sollte d.h. wir müssen in chronischen Experimenten versuchen festzulegen, bis zu welchem Zeitpunkt überhaupt mit einer Reinnervation nach Wiederherstellung des peripheren Recurrensaneurons in dieser oder jener Methodik zu rechnen ist. Wir werden demnächst Untersuchungen aufnehmen.

## DISCUSSION

*U Portmann* Avez vous une expérience de cette chirurgie chez l'homme? Car j'ai eu l'occasion durant une thyroïdectomie de faire une suture récurrente chez l'homme avec un excellent résultat fonctionnel final.

*H H Naumann* 2 Fragen (1) Wie sind die anatomischen Verhältnisse bezüglich Vagus und Recurrent-Bündel beim Menschen? Gestattet es die anatomische Situation eventuell auch beim Menschen derartige Anastomosen anzulegen? (2) Wie ist im Experiment am Hund die Funktion des Stimmbandes in Bezug auf die Synchronisation mit der gesunden Seite und in Bezug auf die Qualität der Stimme (d. h. des Sprechens)?

*H Engström* It has been shown recently by Rune Stenborg that all motor end plates disappear after a complete sectioning of the recurrent nerve. The final evidence of a regeneration would be the reappearance of motor end plates. Have you looked upon this?

*M Arslan* The proprioceptive function plays a very important part in the recurrential function. Would it be possible to testify this fact in your very interesting experiments?

*F Brunetti* Je voudrais demander si par hasard on a essayé de faire des épreuves pharmacologiques avec les curares pour contrôler le différent comportement électromyographique des activités musculaires et adductives dans la régénération nerveuse.

*J Jeschek* Wenn man auch Ergebnisse von Tierexperimenten nicht ohne Weiteres auf den Menschen übertragen kann so haben die interessanten Untersuchungen von Miehke wieder bestätigt dass nach Durchschneidung der Nervus recurrens eine Paramedianstellung des Stimmbandes erfolgt. Dieser Befund stimmt mit unseren Ergebnissen der Nervendurchschneidungen beim Menschen überein.

Dann möchte ich noch fragen, ob man beim Menschen den V. recurrens ebenfalls so exakt vom Vagus abpräparieren kann. Weiter haben Sie versucht im Recurrensbündel des Vagus die Abduktions- und die Adduktionsfasern zu trennen? Ich glaube, dass bei einer Stenose der Glottis mit Paramedianstellung der Stimmbänder es vor allem wichtig wäre die Abduktionsfasern des Vagus mit dem distalen Recurrensstumpf zu verbinden.

*L B W Jongkees* Have you also been doing chronic experiments, i.e. graftings of the recurrent nerve some days or weeks after section of the nerve?

*J Angel-James* The success of this experiment may depend upon the shortening of the nerve regenerating pathway. Suture of the nerve in the neck was performed in a soldier wounded by a small fragment of shell. The inner chord of the vagus nerve was cut cleanly at the level of 6th C. Vertebra with immediate loss of voice. At operation 2 weeks later the shell fragment was removed from the nerve in which it was embedded. The cut ends were freshened and sutured easily end-to-end. The voice recovered but when the larynx was examined 3 years after the war it was found that the vocal cord was still paralyzed.

*A Miehke* (Antwort) an Hr. Portmann und Hr. Naumann Es ist wichtig zu wissen dass Herr Portmann ebenso wie Zöllner, Doyle und andere mit der direkten Vereinigung des durchtrennten V. recurrens mit Hilfe der einfachen Naht zu einer Funktionswiederkehr der Stimmklappen gelangt sind. Die Wieder-



Abb. 1



Abb. 2

Abb. 1 Gansensergelparrotte rechts nach thorakaler L. Verletzung des Vagus und des Recurrens durch Tumor

Abb. 2 Xagus links über dem Abgang des Vagus recurrens durch Tumor vollkommen zerstört (siehe Pl. I)

der Recurrens- und Vagusanschaltung beobachtet wurde würde der experimentell nachgewiesenen Durchschneidung des Vagus recurrens und des R. externus des Vagus laryng superior oder einer Durchschneidung des Vagus bei dem Abgang des Vagus laryng superior entsprechen

Somit wäre im letzten Fall durch die totale Leitungsunterbrechung des Vagus und des Recurrens eine retrograde motorische Schwächung des oberen Kehlkopfnerven eingetreten, so daß durch Nachlassen der Spannung des Musculus cricothyreoideus eine schlaffe Intermediärstellung des betroffenen Stimmbandes eintreten ist. Da es sich bei diesen Fällen um langsam wachsende Tumoren des Oesophagus und der Bronchien, um eine Struma maligna und um ein langsam sich ausweitendes Aneurisma der Aorta gehandelt hat, wurde von Hofer angenommen, daß diese retrograde zufällige Funktionschwächung des Stimmbandes durch eine atrophische Degeneration der Ganglienzellen im Nucleus ambiguus hervorgerufen wird. Der Sitz dieser beschriebenen zufälligen retrograden Störung im Bereich des oberen Kehlkopfnerven würde somit im motorischen Stammganglion, also bulbär bedingt sein, während der Ausfall des Recurrens peripher lokalisiert ist.

Auf der Suche nach anderen solchen retrograden Funktionsstörungen im Vagusbereich haben wir an der Grazer Klinik und an meiner eigenen Ab-

## ZUM PROBLEM DER RETROGRADEN MOTILITÄTSSTÖRUNGEN IM VAGUSBEREICH

J. JESCHEK

Leoben Österreich

Die Beobachtungen nach Nervendurchschneidungen und Tumorerstörungen des Vagus und seiner motorischen Äste haben erwiesen, daß nicht nur distal der Leitungsunterbrechung, sondern auch proximal derselben motorische Ausfälle auftreten können. So führten Läsionen des Vagus unter dem Abgang der Rr. pharyngei zu Paresen des Gaumensegels und es traten schlaffe Lähmungen der Stimmbänder in Intermediärlähmung auf, wenn der N. recurrens und der N. vagus unter dem Abgang des Recurrens unterbrochen waren, während bei Unterbrechung des Recurrens allein eine Paramedianlähmung auftritt. Auch nach tiefer thorakaler und abdominaler Vagotomie konnten retrograd oft wechselnde Störungen der Motilität und der Koordination der Bewegungen des Gaumensegels festgestellt werden. Es wird angenommen, daß im Vagus der Ausfall sensibler Potenzen den Tonus segmentär höher innervierter Muskeln herabsetzen kann oder bei längerer Leitungsunterbrechung retrograde Ganglienzelldegenerationen im Nucleus ambiguus eine ähnliche Wirkung auslösen.

Unter retrograden Motilitätsstörungen verstehen wir motorische Ausfälle, die nicht distal einer Nervenläsion, sondern proximal einer solchen auftreten. Erstmalig hat Mann (1904) die Vermutung ausgesprochen, daß die kombinierten Lähmungen von Stimmband und Gaumensegel bei thorakaler Unterbrechung durch rückläufige Degeneration der Nervenfasern und Übergreifen auf den Plexus pharyngeus bedingt sein können. Auch ein rückläufiger Reflexvorgang vom Recurrens ausgelöst, wird von ihm in Erwägung gezogen. Im Jahre 1944 konnte Hofer auf Grund von 4 obduzierten Fällen nachweisen, daß bei Zerstörung des N. recurrens und bei kompletter Ausschaltung des N. vagus unter dem Abgang des N. recurrens eine schlaffe Stimmbandlähmung in Intermediärlähmung auftritt, während bei Zerstörung des N. recurrens allein ohne Vagusausschaltung (3 Fälle) eine Lähmung in Paramedianlage zu beobachten war. 1964 wurden mit Hofer gemeinsam noch weitere 7 obduzierte Fälle mit Recurrens- und Vaguszerstörung in Intermediärlähmung veröffentlicht. Die Paramedianlage bei Recurrensausschaltung entspricht den Ergebnissen der mit Hofer gemeinsam mehrfach durchgeführten operativen Durchschneidung des N. recurrens am Menschen. Die schlaffe Intermediärlähmung, die bei der Kombination



Abb. 1

Abb. 2

Abb. 1 Os oesophageale rechts nach überakuter Unterbrechung am Vagus und V. recurrentens d. rech. T. mor.

Abb. 2 Vagus unter dem Abgang des V. recurrentens d. rech. T. mor. (Knochen zerstört (siehe Pf.))

der Recurrens- und Vagusauschaltung beobachtet wurde, würde der experimentell nachgewiesenen Durchschneidung des V. recurrentens und des R. externus des V. laryng. superior oder einer Durchschneidung des Vagus ober dem Abgang des V. laryng. superior entsprechen.

Somit wäre im letzten Fall durch die totale Leitungsunterbrechung des Vagus und des Recurrens eine retrograde motorische Schwächung des oberen Kehlkopfnerven eingetreten so daß durch Nachlassen der Spannung des Musculus cricothyroideus eine schlaffe Intermediärstellung des betroffenen Stimmbandes eingetreten ist. Da es sich bei diesen Fällen um langsam wachsende Tumoren des Oesophagus und der Bronchien, um eine Strumamaligna und um ein langsam sich anwetzendes Aneurysma der Aorta gehandelt hat wurde von Hofer angenommen, daß diese retrograde zurückliegende Funktionschwächung des Stimmbandes durch eine atrophische Degeneration der Ganglienzellen im Nucleus ambiguus hervorgerufen wird. Der Sitz dieser beschriebenen zurückliegenden retrograden Störung im Bereich des oberen Kehlkopfnerven würde somit im motorischen Stammganglion, also bußbar bedingt sein, während der Ausfall des Recurrens peripher lokalisiert ist.

Auf der Suche nach anderen solchen retrograden Funktionsstörungen im Vagusbereich haben wir an der Grazer Klinik und an meiner eigenen Ab-



tellung durch Jahre hindurch die kombinierten Lähmungen von Stimmband und Gaumensegel beobachtet und gesammelt. So konnten wir von 21 Lähmungen des N. vagus, 12 Fälle kombiniert mit Gaumensegelparese bei thorakaler Vagusschädigung gemeinsam mit Hofer im Jahre 1964 veröffentlichen (Abb. 1). Bei 11 von diesen Patienten war eine einseitige Stimmbandlähmung in Intermediärstellung und eine Gaumensegelparese und bei einem nur eine Gaumensegelparese allein vorhanden. Bei 4 Fällen, bei welchen bereits längere Zeit ante exitum eine einseitige Gaumensegelparese und Stimmbandlähmung in Intermediärstellung festgestellt werden konnte, hatten wir auch Gelegenheit bei der Obduktion eine genaue Lokalisation der schuldigen Noxe festzustellen. Bei allen 4 Fällen war es durch destruisierende Prozesse zu einer kompletten Leitungsunterbrechung im N. vagus und im N. recurrens gekommen. Bei diesen Fällen kann somit die Gaumensegelparese nur durch einen retrograden Vorgang erklärt werden, da die Unterbrechung im thorakalen Anteil des Vagus erfolgt ist.

Aber nicht nur bei solchen langsam progredienten Zerstörungsprozessen kann es zu einer solchen retrograden Motilitätsstörung kommen, sondern auch unmittelbar nach einer operativen Durchtrennung des N. vagus. So hat Messerklinger (1949) einen Fall veröffentlicht, bei dem vor der Operation einer malignen Struma eine normale Beweglichkeit des Gaumenbogens und der Stimmbänder nachgewiesen war und bei dem nach Durchtrennung des N. vagus in Ringknorpelhöhe eine schlaffe Lähmung des Stimmbandes in Intermediärstellung und eine Gaumensegelparese auftrat. Einen zweiten Fall einer kombinierten Lähmung nach operativer Vagusdurchschneidung konnte ich beobachten, nur war das Stimmband zwar schlaff, auch etwas exkaviert, doch in einer Stellung zwischen Paramedian und Intermediär. Die Stimme aber war hochgradig heiser, fast aphonisch wie sonst nur bei der Intermediärlähmung und im Stroboskop konnte man über die Medianlinie überschlagende flatternde Schwingungen, wie wir sie nur bei vollkommen schlaffen Stimmbändern sehen, feststellen.

Außer diesen beschriebenen Fällen einer retrograden Störung im Bereiche des oberen Kehlkopfnerven und im Versorgungsgebiet des Gaumenbogens nach Ausschaltung des Recurrens und des N. vagus hatten wir auch Gelegenheit eine retrograde Motilitätsstörung des Gaumenbogens nach totaler Leitungsunterbrechung des N. vagus allein unter dem Abgang des N. recurrens zu beobachten. An dem bei der Obduktion gewonnenen Präparat kann man deutlich die komplette Zerstörung des Vagus unter dem Abgang des Recurrens sehen (Abb. 2), während der obere Vagus und Recurrens vollkommen intakt ist. Bei dieser Patientin konnten wir bei mehreren Untersuchungen eine Gaumensegelparese rechts bis kurz vor dem Tod bei normal beweglichen Stimmbändern nachweisen. Auf Grund dieses Befundes und unserer langjährigen Erfahrungen mit solchen Motilitätsstörungen haben wir bereits vor dem Exitus eine völlige Leitungsunterbrechung des rechten N. vagus kaudal vom Abgang des N. recurrens angenommen.

In letzter Zeit habe ich nun mit Stauber (1968) gemeinsam Gelegenheit



Abb. 3. Gaumensegelparvas rechts nach orderer tr. Lufärer Vagotomie.

gehabt, 14 Patienten, bei denen wegen Ulcusbeschwerden eine Vagotomie durchgeführt wurde laryngologisch längere Zeit zu beobachten. Die verschiedenen Arten der Vagotomie erfolgten in Hiatunähe. Einmal wurde eine beidseitige thorakale 3mal eine beidseitige abdominale trunkuläre, 4mal eine abdominale trunkuläre vordere 1mal eine abdominale trunkuläre hintere und 5mal eine abdominale totale Vagotomie bei Gastrektomie durchgeführt. Bei diesen Patienten wurde nun am Tag nach der Operation beginnend, und bei 8 entscheidenden Testfällen bereits vor der Operation ein genauer neurologischer Pharynx und Larynxstatus erhoben. Bei keinem dieser Patienten konnten Motilitätsstörungen an den Stimmbändern festgestellt werden. Wohl aber wurden bei 13 Fällen oft wechselnde Motilitätsstörungen des Gaumenbogens bei Phonation, bei Respiration und beim Würgereflex beobachtet. Bei 8 Fällen war eine Asymmetrie der Gaumensegelbewegung vorhanden, die bei aufeinanderfolgenden Untersuchungen einmal nach rechts und einmal nach links gerichtet war. Manchmal sahen wir kreisende Bewegungen, so daß das Gaumensegel einmal nach der einen und dann nach der anderen Seite verzogen wurde. In diesen Fällen haben wir es vermieden von einer Paræse zu sprechen, sondern uns darauf beschränkt, die jeweilige Seitenverschiebung bekannt zu geben. Es dürfte sich dabei um wechselnde retrograde Tonusüberhebungen der Gaumenmuskulatur und zum Teil um Koordinationsstörungen der Innervation des beidseitigen Einsatzes handeln. Bei 5 Patienten aber sahen wir eine bleibende zum Teil mehrere Wochen lang beobachtete Seitenverschiebung. Wir

sind der Meinung daß wir in diesen Fällen von einer partiellen Parese sprechen können (Abb 3). Wir fanden 2 Paresen des rechten Gaumenbogens bei totaler Vagotomie eine Parese des linken Gaumenbogens und eine Parese des rechten Gaumenbogens bei vorderer trunkulärer Vagotomie und schließlich eine Parese des rechten Gaumenbogens bei der einzigen von uns durchgeführten hinteren Vagusdurchtrennung. Diese sicherlich nur retrograd zu erklärenden Bewegungsstörungen des Gaumenbogens treten nun nicht wie bei den distalen Lahmungen in der Regel immer an der Seite des unterbrochenen Vagusanteiles auf eine Beobachtung die auf Grund der anatomischen Struktur des N. vagus im Hiatusbereich leicht zu verstehen ist. Denn beide Vagi bilden ober dem Hiatus einen Plexus mit zahlreichen Anastomosen untereinander. Die Äste des linken Vagus mit den Zweigen des rechten vereinigen sich zu einem Truncus, der an die Vorderseite des Ösophagus zieht während auf gleiche Weise gebildet der Truncus des rechten Vagus an die Hinterfläche des Ösophagus gelangt. Somit haben beide Trunci in Hiatusnahe Nervenfasern vom rechten und linken Vagus. Eine Seitenbeeinflussung des Gaumensegels würde also von der individuellen Variante der Anastomosenbildung abhängen. Schwer zu erklären wäre nur wieso bei 3 totalen Vagotomien nach Gastrektomie 3mal eine Parese des rechten Gaumenbogen aufgetreten ist.

Eine beiderseitige Einschränkung der Gaumenbogenbeweglichkeit sahen wir 5mal. 3mal bei Gastrektomie und beidseitiger Vagotomie entsprechend der beidseitigen Nervenstörung 1mal bei einer vorderen und einmal einer hinteren trunkulären Vagotomie kombiniert mit einer Seitenverschlebung.

Eine weitere Form der Motilitätsstörung, die wir sonst nur bei zentralen Ausfällen im Vagusbereich zu sehen gewohnt sind, wurde bei 4 Fällen beobachtet, nämlich eine Koordinationstörung der automatisch gesteuerten Gaumensegelbewegungen bei Ein- und Ausatmung, bei Phonation und beim Würgereflex. Man hatte den Eindruck als wüßte das Gaumensegel nicht mehr wie es sich bei seinen verschiedenen Funktionen zu verhalten hat. So wurde z.B. das Gaumensegel in zwei Phasen bei der Phonation kontrahiert, blieb dann aber auch bei der Atmung in dieser Phonationsstellung und es kam erst zur Entspannung wenn der Mund geschlossen wurde. Diese eigenartigen Koordinationstörungen betrafen auch die respiratorischen Bewegungen. Während normal bei der Inspiration das Gaumensegel gesenkt und bei Expiration gehoben wird traten gegenteilige paradoxe Bewegungen auf. Bei 7 Fällen wurden Zuckungen des Gaumenbogens bei Phonation wie auch bei Entspannung beobachtet.

Aber nicht nur im motorischen Bereich sondern auch im sensiblen wurden bei der tiefen Vagotomie retrograde Beeinflussungen festgestellt. So fanden wir bei 10 von 14 Fällen eine Verminderung des Würgereflexes. Diese Tatsache ist vielleicht am leichtesten erklärbar da der sensible Anteil des Reflexbogens, in dem die Cardia und die Organe des Abdomens liegen und in dem auch der Gaumenbogen einbezogen ist unterbrochen wurde. Nervöse Schluckstörungen wurden nicht beobachtet.

Diese erwähnten motorischen und sensiblen Ausfallserscheinungen waren in den ersten Tagen nach der Operation am stärksten ausgeprägt, zum Teil veränderten sie sich, zum Teil nahmen sie nach wenigen Wochen ab, oder verschwanden. Bei 2 Patienten, bei denen allerdings keine Voruntersuchung stattgefunden hatte, waren auch nach 20 Monaten bzw. 3 1/2 Jahren deutliche Abweichungen der Gaumensegelmotorik zu sehen.

Die Tatsache, daß bei solchen thorakalen und zum Teil abdominalen Vagusauschaltungen segmentär höher gelegene Nervenäste funktionelle Ausfälle aufweisen, ist wohl auf Grund dieser genau lokalisierten Nervendurchschneidungen und zum Teil auch antroptisch verifizierten thorakalen Destruktionsprozessen nicht mehr abzuleugnen. Es fragt sich nur, wie man diese Beobachtungen erklären kann. Um diesem retrograden Phänomen näherzukommen, müssen wir berücksichtigen, daß der V. vagus kein rein motorischer Nerv ist, sondern afferente sensible Fasern, afferente sensorische (Geschmacksfasern), efferente motorische Fasern sympathische und schließlich autonome dem Parasympathicus angehörige Neuriten enthält. Da außerdem in den Kehlkopfmuskeln von verschiedenen Autoren (Brookhurst & Edgeworth, 1940 Goerttler 1950 Paulsen, 1958 König & von Leden, 1961 Lucas Keene, 1961 Rudolph, 1956) Muskelspindeln nachgewiesen wurden, von Molinari (1962) auch elektrophysiologisch bestätigt werden konnte, muß auch das System der efferenten kleinkalibrigen Nervenfasern vorhanden sein, das den Grad der tonischen Spannung bestimmt, indem es die für die reflektorische Tätigkeit entscheidende afferente Entladung der Muskelspindeln moduliert. Der Tonus im Warmblütermuskel wird vom Zuckungssystem der großkalibrigen motorischen Fasern derart hervorgerufen, daß ständig einzelne Muskelfasern sich in unmerklicher Kontraktion befinden. Somit also ist der Tonus des Warmblütermuskels, wie experimentelle Untersuchungen von Granit (1955) und Rudolph (1956) nachweisen, weitgehend von den afferenten Impulsen abhängig. So meint Rudolph (1956): „Der Muskel steuert die Erregbarkeit des Motoneurons durch seine sensiblen Impulse selbst.“ Dieser Mechanismus wird eine umso größere Rolle spielen, je differenzierter die Arbeitsweise eines Muskels ist, so etwa bei den Blickbewegungen eines Auges und wahrscheinlich auch bei den differenzierten Bewegungen der Kehlkopfmuskeln, die im Dienste der Stimmbildung stehen. Überdies sind sämtliche motorische Funktionen der inneren Kehlkopfmuskeln, aber auch des Gaumenbogens und des Rachens, wie Molinari (1962) experimentell nachgewiesen hat, nicht nur von ihren zugehörigen motorischen Nerven, sondern von Nervenästen verschiedener Nerven abhängig, soweit sie eben in einem tonischen Reflexsystem einbezogen sind. Wir sind daher der Meinung, daß die plötzliche Abschaltung einer größeren Anzahl qualitativer Impulse, wie es bei der Vagusdurchschneidung der Fall ist, eine reflektorische Herabsetzung des Tonus in den jeweils betroffenen Reflexbogen zur Folge haben kann.

Am Gewölbe des weichen Gaumens mit den beiden freihängenden Gaumenbögen und der Uvula, werden feinste Abänderungen des Tonus sich

leicht auswirken und sind auch gut zu beobachten. Viel schwieriger aber wird der Nachweis einer Schwächung oder eines Ausfalles der motorischen Aktivität des oberen Kehlkopfnerven gelingen, da dies nur durch Feststellung einer herabgesetzten Spannung der Stimmbänder möglich ist. Diese wird allerdings wenn auch eine Recurrenslähmung vorhanden ist symptomatisch als Intermediärstellung des Stimmbandes in Erscheinung treten, wenn keine paralytische Kontraktur den Positionswechsel von paramedian nach intermediär verhindert. Während wir somit bei einer plötzlichen Leitungsunterbrechung des Vagus und seiner Äste die motorischen retrograden Ausfälle durch Funktionsstörung im zugehörigen Kerngebiet, in Folge Ausschaltung größerer sensibler Zuzugsgebiete erklären können, wird bei längerem Bestand einer solchen Lähmung dafür zuzüglich die von Hofer (1944) nachgewiesene retrograde tigrolvtische Degeneration der Ganglienzellen im Nucleus ambiguus mitbeteiligt sein.

### RESUME

Les observations après section de nerfs et de destructions du nerf vague et de ses rameaux moteurs causées par des tumeurs ont prouvé que non seulement distalement de l'interruption de conduction mais aussi à proximité de celle-ci, des troubles moteurs peuvent se produire. Après une profonde vagotomie thoracique et abdominale telle qu'elle est effectuée à l'occasion d'un ulcère gastrique on a également pu constater des troubles moteurs retrogrades du voile du palais.

### SUMMARY

Observations carried out after the dissection of nerves and the destruction of the vagus and its branches by tumours, have proved that motorial deficiencies cannot only occur distal to the interruption of motility but also proximal to it. Thus, lesions of the vagus below the branching of the pharyngeal ramus resulted in the paresis of the soft palatum, and flaccid paralysis of the vocal cords in the intermediate position occurred when the nervus recurrens and the nervus vagus were interrupted while the interruption of the nervus recurrens alone resulted in a paramedian paralysis. Retrograde changing disturbances in motility and in the coordination of soft palatum motions could often be determined after deep thoracic and abdominal vagotomy. It is supposed that the loss of sensory energy in the vagus can reduce the tension of segmentally higher activated muscles, or that in cases of longer interruptions of motility retrograde degenerations of ganglionic cells in the nucleus ambiguus can produce similar effects.

### LITERATUR

- BLANCKOHL, R., und MOLINARI, G. 1962. Electron micrographic evidence of small spindles and other sensory endings in the intrinsic laryngeal muscles of the cat. *Acta Otolaryng.* (Stockh.) 55: 253.

- BROOKHURST, R. J. und EDGEMORTH, F. H., 1940: The fibre components of the laryngeal nerves of *Macaca M. latia*. *J Anat* 75 358.
- GOETTLER, K., 1950: Die Anordnung, Histologie und Histogenese der quergestreiften Muskeln im menschlichen Stimmband. *Z Anat Entwicklungsgesch* 115, 352.
- GRAY II, R., 1955: *Receptors and sensory perception* Yale University Press, New Haven und London.
- HORST, G., 1944: Zur motorischen Innervation des menschlichen Kehlkopfes. *Z Ges Neurol Psychiat* 177 183.
- HORST, G., und JASCHKE, J., 1940: Die Lähmung des Nervus recurrens beim Menschen. *Arch. Otorhinolaryng* 45, 301.
- 1944: Der Nervus vagus und die motorische Innervation. *Misch. Ohr Akustik* 98 193.
- JASCHKE, J., 1953: Theorie und Klinik der Stimmbandlähmung. *Arch. Ohr Nas Kehlkopfheilk*, 162 227.
- 1954: Die Gaumensegelparese — Symptom einer thorakalen Vagusläsion. *Misch. Otorhinolaryng* 160 223.
- JASCHKE, J. und SCHNEIDER, R., 1958: Zur Frage der Dysfunktionen des Gaumensegels und Rachen nach Vagusläsion im Halsbereich. *Chirurg* 39 512.
- KOHN, W. F. und LUDWIG, H., 1961: The peripheral nervous system of the human larynx. II. The thyroarytenoid (vocalis) muscle. *Arch. Otolaryng. (Chic.)* 74 133.
- LICK KERN, M. F., 1961: Muscle spindles in human laryngeal muscles. *J Anat* 95 23.
- MAY, M., 1904: Über Gaumenlähmung. *Z. Ohrenheilk.*, 47 82.
- METZGER, W., 1949: Der Nervus vagus und die Gaumensegelinnervation. *Proc. Int. Congr. Otolaryng.* (London).
- MOLINARI, G. A., 1947: Sulla partecipazione delle fibre afferenti del nervo laringeo ricorrente al riflesso della deglutizione. *BoU Med. Orecch* 80 665.
- PETER, K., 1938: Über Vorkommen und Zahl von Muskelspindeln in den Kehlkopfsmuskeln des Menschen (*M. cricoarytenoideus dorsalis*, *M. cricothyroideus*). *Z. Zellforsch.*, 44 349.
- RÖDLER, G., 1936: Neuere Anschauungen über die funktionelle Verknüpfung von Muskel- und Nervensystem. *Anales Univers. Saratensis Med* 4 2.

FRANK JOSEFSTRASSE 14  
LIEBEN, ÖSTERREICH

## THE INFLUENCE OF HEAT ON THE UPPER RESPIRATORY TRACTS

B GUŠIĆ, Z KRAJINA, Z POLJAK, V KONIĆ-CARNELUTTI and I BABIĆ

*From the ENT-Clinic and the Pediatric Clinic Medical Faculty University of Zagreb Zagreb Yugoslavia*

The authors examined the upper respiratory tracts of workers in an electric light bulb factory who were exposed to heat. Clinical, bacteriological and histological examinations were made. Parallel examinations were made with albino rats which were exposed to heat during various lengths of time.

Heat is transferred from the environment into the body by the physical processes of radiation, conduction, and convection. Radiation, conduction, convection, and evaporation are the avenues for heat loss from the body. About 12% of the body's heat loss is normally dissipated by evaporation of water from the lungs with the warmed exhaled air. Excessive heat harms the shocked organism in several ways:

1. It produces a widespread superficial vasodilatation and distribution of blood to nonessential parts of the body and compensatory constriction in the splanchnic area.

2. Excessive heat increases the metabolism and the oxygen requirement of tissues. Overheating the patient necessitates the utilization of more oxygen and hence leads to a more severe anoxemia, which increases capillary damage and capillary permeability.

3. Excessive heat is likely to increase perspiration and consequently promote dehydration.

Increased rates of sweating, decreased concentration of salt in the sweat, altered sensitivity of the thermoregulatory center and increased blood volume are the various responses with which acclimatization to heat is explained.

Physiological disturbances due to heat stress are evaluated in terms of the body temperature rise, increased cardiovascular demands, diversion of blood to the skin from other organ systems, sweat secretion and water and electrolyte depletion and fatigue. In all water-dwelling animals the nasal mucous membrane is the chief mechanism for the loss of heat; hence the complexity of the maxillary turbinates which greatly increase the surface area of the nasal mucous membrane. The reduction of the ethmoidal max

This work was supported by the Council of Scientific Work of Croatia.

lary and nasal turbinates in man and the higher primates is probably a factor concerned in the transformation of the head and the relation between respiration and olfaction. The degree of heat loss taking place through the nasal mucous membrane depends upon the vascularity, moisture and the area of the mucous membrane. In man the area of the nasal mucosa is reduced as the skin becomes a more efficient mechanism for the regulation of heat loss, but there is nevertheless a complex functional correlation between the nasal mucous membrane and skin which is controlled by the autonomic nervous system and certain ductless glands. In this way we can explain the noxious effect of heat on the upper respiratory tracts by a two-fold mechanism. The effect of heat itself on the mucous membrane and the reflectory mechanism of the skin—the mucous membrane of the nose.

### *Our Investigations*

Our investigations regarding the influence of heat on the respiratory mucous membrane were made experimentally on rats and by clinical examination of the workers of an electric light bulb factory who working 8 hours a day were exposed to temperatures from 30 to 51.5 C. As we had no experience of the behaviour of rats at high temperatures (and could find no references in the literature) we chose different temperatures, beginning with 40 C during various nights of time. Rats having no sweat glands, their general reaction is much more pronounced, a fact we had to take into consideration. These investigations consisted of histological studies of the nose and trachea.

#### *(a) The findings in the nose and pharynx*

The mucous membrane of the nose was dry, glossy and pink. In the nasal vestibule there was dried up secretion. In eight cases there were initial atrophic changes of the posterior part of the nose bilaterally, in three cases unilaterally. In one case there was a typical picture of ozena. In 27 cases the mucosa of the pharynx was dry with accumulated desiccated secretion and granular formations.

#### *(b) The diseases of the respiratory tract in the course of duration of work*

Frequent colds	27 cases	Bronchitis	3 cases
Asthma	20 cases	Pneumonia	2 cases
Sore throat	9 cases	Bleeding of the nose	2 cases
Aliment	3 cases		

#### *(c) Other diseases in the course of the duration of work*

Headaches	17 cases
Psychoneurosis	10 cases



## THE INFLUENCE OF HEAT ON THE UPPER RESPIRATORY TRACTS

B GUŠIĆ, Z KRAJINA, Z POLJAK, V KONIĆ-CARNELUTTI and I BABIĆ

*From the ENT-Clinic and the Pediatric Clinic Medical Faculty University of Zagreb Zagreb Yugoslavia*

The authors examined the upper respiratory tracts of workers in an electric light bulb factory who were exposed to heat. Clinical, bacteriological, and histological examinations were made. Parallel examinations were made with albino rats which were exposed to heat during various lengths of time.

Heat is transferred from the environment into the body by the physical processes of radiation, conduction, and convection. Radiation, conduction, convection, and evaporation are the avenues for heat loss from the body. About 12% of the body's heat loss is normally dissipated by evaporation of water from the lungs with the warmed exhaled air. Excessive heat harms the shocked organism in several ways:

- 1 It produces a widespread superficial vasodilatation and distribution of blood to nonessential parts of the body and compensatory constriction in the splanchnic area.

- 2 Excessive heat increases the metabolism and the oxygen requirement of tissues. Overheating the patient necessitates the utilisation of more oxygen and hence leads to a more severe anoxemia, which increases capillary damage and capillary permeability.

- 3 Excessive heat is likely to increase perspiration and consequently promote dehydration.

Increased rates of sweating, decreased concentration of salt in the sweat, altered sensitivity of the thermoregulatory center, and increased blood volume are the various responses with which acclimatisation to heat is explained.

Physiological disturbances due to heat stress are evaluated in terms of the body temperature rise, increased cardiovascular demands, diversion of blood to the skin from other organ systems, sweat secretion, and water and electrolyte depletion and fatigue. In all water-dwelling animals the nasal mucous membrane is the chief mechanism for the loss of heat, hence the complexity of the maxillary turbinates which greatly increase the surface area of the nasal mucous membrane. The reduction of the ethmoidal max

This work was supported by the Council for Scientific Work of Croatia.

TABLE 1 *Experimental animals (31 albino rats) Histological findings of the mucosae of the nose and the trachea*

Group I (Figs. 1 and 2) Three days of 7 hours exposure to 40°C. Six animals

Nose Mucosa changed the superficial epithelium, strong glandular hyperplasia and increased intracellular edema, diffuse infiltration, sporadic bleeding

Trachea Small infiltrates in perimembranous three times, normal findings in alveoli

Group II (Figs. 3 and 4) Twenty days of 3 hours exposure to 30°C. Five animals

Nose Changed epithelium with moderate infiltrates in the mucosa twice, bleeding in the mucosa with stronger glandular hyperplasia twice, normal findings once

Trachea Minor infiltrates in the mucosa with enlarged gland four times. Mild inflammation of the mucosa once

Group III (Figs. 5 and 6) First week 3 hours exposure per day at 30°C. Second week 3 hours exposure per day to 40°C. Third week 4 hours exposure per day at 40°C. Fourth week 4 hours exposure per day at 40°C. Eight animals

Nose Changed epithelium, glands changed and disintegrated, enlarged blood vessels with bleeding and inflammatory infiltrates

Trachea Changed epithelium in lesser degree with mucosa surface subepithelially enlarged blood vessels, oedema, sporadic bleeding, and smaller inflammatory infiltrates

Group IV (Figs. 7 and 8) Thirty-one days of 7 hours exposure to 30°C. Seven animals

Nose The changes of the epithelium, glands changed, in some places degenerated, bleeding in the mucosa well pronounced inflammatory infiltrates

Trachea Changed epithelium in lesser degree, in one case papillary hyperplasia of the epithelium, infiltrates in perimembranous, sporadic bleeding

Group V (Figs. 9 and 10) Fifteen days of 7 hours exposure to 40°C. Fifteen days of normal conditions. Two animals

Nose Epithelium regular glands very swollen in the stage of recovery associated with infiltration moderate

Trachea Slight infiltrates in perimembranous with enlarged gland

### (f) Bacteriological findings

#### I

Potentially pathogenic flora

Potentially pathogenic flora and saprophytic flora

Saprophytic flora

Sterile

23

0

44

0

#### II

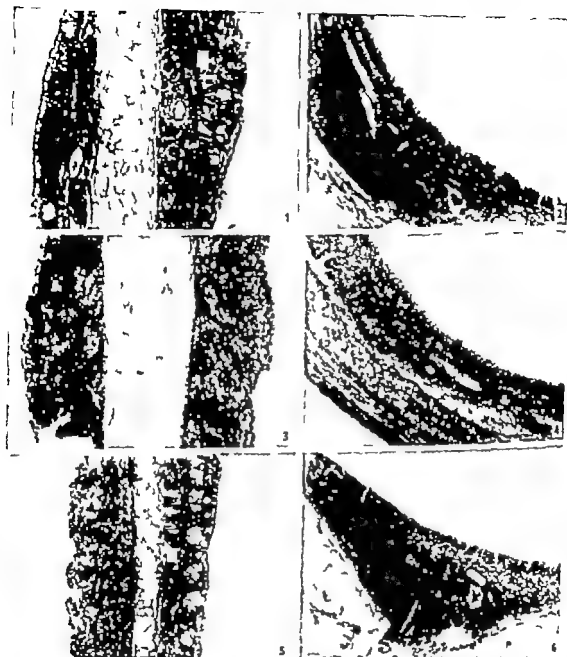
Potentially pathogenic flora

Saprophytic flora

STA P III PH E STH C

STA SC SC STN STAH II

Act also large 6"



(d) X ray of the sinuses (46) workers

Negative finding	8 case
Positive finding	40 case

(e) Smear of the nose

Mucin	2 cases
Epithelial cells	28 cases
Leucocytes	50 cases
Eosinophiles	15 cases
Microorganisms	18 cases

TABLE 2

Examined workers of the electric light bulb factory  
exposed to the following conditions:

1. The air temperature of working premises	27-35 C
2. Global temperature	33-35.5 C
3. Relative humidity	37-55%
4. The velocity of air streaming, 87 workers	Up to 0.01 m/sec
Women workers: 71	Age 19-48
Men: others 16	Duration of work 1-18 yrs

TABLE 3

Bacteriological control group of healthy rats (120)

Year since	
Infl.	54 cases
Pathogenic	6 cases
Saprophytic	33 cases
Saprophytic + pathogenic	8 cases

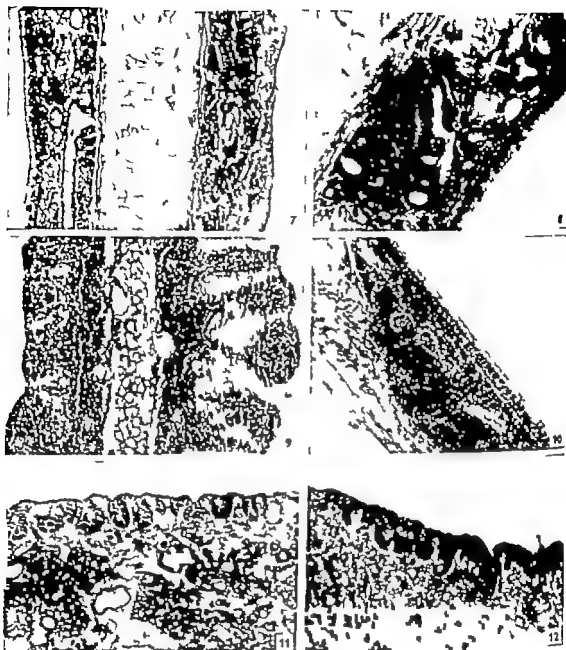
(g) *Pathohistological findings of the nasal mucosa in 12 cases*

There was an alteration of the superficial epithelium leading to a squamous form, an enlarged basal membrane vasodilatation and oedema and, in one case, fibrous changes, numerous glands, and microcellular infiltration (three cases)

(h) *Electrophoresis of proteins was made in 30 cases*

Twenty cases were exposed to a temperature above 35 C and ten cases were exposed to a temperature up to 35 C. In all cases the values of the proteins and gammaglobulins were normal.

We have found histologically that the nasal mucosa reacts to heat as well as other external noxae, and ranges within the frame of the reaction of its compound elements. The change of the epithelium up to the creation of a squamous form, which, in the normal condition of the nasal mucous membrane returns again to its cylindrical form. Hyperplasia of mucous glands and the increase of goblet cells on the surface is a satisfactory reason for the increased necessity to produce mucous. The glands did not decrease numerically even at higher temperatures, but disintegration processes took place which led to the disfunction and degeneration of glands. Oedema and vasodilatation are always present, while fibrosis was less frequently met with (as with human beings) we suppose that vasodilatation by heat is a more constant form which prevents the formation of fibrous tissue. In cases where the rats were given normal conditions, as early in the course of a



# *Explanation*

STA + - *Staphylococcus pyogenes aureus*  
 coagulase +  
 P - *Pneumococcus*  
 HI - *H. mophyly* infl enzao  
 PR - *Proteu*  
 E - *Enterococcus*  
 STH - *Streptococcus haemoliti* us  
 C - *Candida*

STA - - *Staphylococcus aureus* and *ibao*  
 coagulase -  
 SN - *Saprophytic N. laxeriao*  
 SC - *Saprophytic undifferentiated cocci*  
 STV - *Streptococcus viridans*  
 STAH - *Streptococcus rhaemolitycus*  
 D - *Diphteroid*

## ZUSAMMENFASSUNG

Die Autoren haben die oberen Atmungswege bei Arbeitern einer Fabrik elektrischer Glühbirnen die der Hitze ausgesetzt werden, untersucht. Es wurden klinische bakteriologische und histologische Untersuchungen unternommen. Parallel wurden ebenfalls Untersuchungen bei Albino-Ratten, die einer Wärme von verschiedener Dauer ausgesetzt waren vorgenommen.

## REFERENCES

- HARRIS, B. A., 1943. Comments on heat problems. *Industrial hygiene Arch. Environ Health (Chic.)* 11: 593.  
SCOTT, J. 1954. Pathology of the nasal mucous membrane. *J. Otol.* 68: 306.  
WATSON, K., 1964. Bodily reaction to high temperature. *J. physiology* 21: 531.

Prof. B. Gusić, ENT-Clinic, Medical  
Faculty University of Zagreb  
Zagreb, Yugoslavia

fortnight the nasal mucous membrane returned to histological normalisation. The changes of trachea were less frequent and to a much smaller degree as we could see with other physical damage to the nasal mucous membrane.

Our examinations of workers also gave interesting data. Anamnestically the workers complained of the frequent appearance of the common cold, sore throat, and angina. Not in a single case did we find a normal mucosa of the nose during the work. The changes ranged from dryness, the infection of the mucosa itself and, less frequently, the beginning of atrophic changes. Only in one case did we find a typical picture of ozaena. These clinical findings correspond to histological analysis of the nasal mucosa of the workers and experimental animals. Bacteriologically we found an increased positive finding of bacterial flora as well as a large percentage of leucocytes in the smear of the nose. These findings prove the susceptibility of the nasal mucosa after the influence of heat upon the bacterial and virus infection, and the possibility of the appearance of colds, angina, and pharyngitis. The changes in the X-ray pictures of the sinuses are very frequent, but only a few workers were treated for sinusitis. We suppose that the mucosa of the paranasal sinuses reacts to the noxa which influences the nasal mucosa, with stronger oedema because of the looser structure of the mucosa of the sinuses. Among general anamnestical data, we found that the workers suffer from headaches and in 10 cases we could clinically establish psychoneurosis. We cannot go into the explanation of the relation between the headaches and damage to the nasal mucosa, but in other cases of dryness of the nasal mucosa we came across appearances of neuralgia and headache. Very probably the reflexes play a certain role here.

Eosinophils in the smear of the nose in as many as 15 cases is probably connected with the greater liberation of histamine in the mucosa damaged by heat.

Accordingly heat influences the mucosa of the nose by producing disturbances of the normal physiological protective function of the nasal mucosa with repercussions upon the paranasal sinuses and pharynx. The principal mechanisms of this disturbance are the changes of the epithellium and nasal mucus, lysosimes as the protectives factors, and oedema of the tissue which slows down the biochemical processes. However here too broad possibilities of regenerative and reparatory abilities of the nasal mucosa come into consideration.

## RESUME

Les auteurs ont examiné les voies respiratoires supérieures chez les ouvriers d'une usine d'ampoules électriques. On a procédé aux examens cliniques, bactériologiques, histologiques. Parallèlement on a examiné de la même manière les rats albinos qui ont été exposés à la chaleur d'une durée variable.

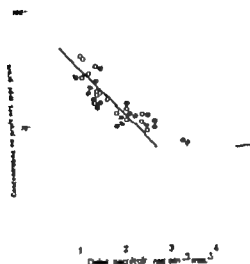


Fig. 1 Relation entre la concentration en protéines et le débit sécrétoire chez les sujets normaux. ● Prélevements réalisés en novembre; ○ prélevement réalisés en juillet et août.

### MATÉRIEL ET MÉTHODES

Le mucus a été prélevé chez deux groupes de sujets.

Le premier groupe est constitué de 9 patients affectés d'une sensibilisation nasale purement périodique aux pollens de graminées. Ces patients présentent chaque année entre mai et juillet, les manifestations cliniques du rhume des foins.

Le second groupe comprend 10 patients dont les crises sont déclenchées exclusivement par des facteurs d'irritation locale d'ordre physique ou chimique. Ces patients qui selon l'expression de Melchior (1950) souffrent d'érythème nasal d'origine exogène ne présentent aucun signe anamnestique clinique ou biologique de sensibilisation allergique.

Le prélèvement du mucus a été pratiqué, comme chez les sujets normaux, à l'aide de fragments rectangulaires de papier filtre placés au contact de la

TABLEAU 1 La composition inorganique du mucus nasal

Dosages réalisés chez 40 sujets normaux.

	Valeurs extrêmes ( $\mu\text{eq g}^{-1}$ )	Valeurs moyennes ( $\mu\text{eq g}^{-1}$ )
Concentration en Na	120,69 et 135,92	127,70
Concentration en Cl	128,83 et 148,99	138,23
Concentration en H	12,37 et 25,01	17,30
Concentration en Ca	3,23 et 6,77	4,54



# ÉTUDE COMPARATIVE DE LA COMPOSITION CHIMIQUE DU MUCUS NASAL PRÉLEVÉ AU COURS DES HYPERSÉCRÉTIONS D'ORIGINE RÉFLEXE ET ALLERGIQUE

J MÉLON

*Clinique Oto-Rhino-Laryngologique Université de Liège Liège Belgique*

Deux composants du mucus subissent des variations de concentration différenciées suivant le type d'hypersécrétion. Il s'agit du K et des glycoprotéines. Au cours des hypersécrétions d'origine réflexe, la concentration en K augmente et la concentration en glycoprotéines diminue tandis qu'au cours des hypersécrétions d'origine allergique la concentration en K diminue et la concentration en glycoprotéines augmente. Le mécanisme de ces modifications est discuté ainsi que leur intérêt diagnostique.

Le mucus nasal prélevé chez des sujets normaux, dans des conditions techniques rigoureuses, présente une composition chimique bien définie. Nous avons montré dans un travail précédent, que les concentrations moyennes des éléments inorganiques, évaluées en microéquivalents par gramme, sont de 127,7 pour le Na, de 138,8 pour le Cl, de 17,3 pour le K, de 4,8 pour le Ca. Les concentrations limites sont indiquées dans le Tableau I. Elles sont constantes, quel que soit le débit sécrétoire mesuré (Mélon & Schioffele, 1966).

Il n'en est pas de même pour la concentration en glycoprotéines qui varie fortement avec la sécrétion pour des débits inférieurs à  $3 \text{ mg cm}^{-2} \text{ min}^{-1}$ . Il existe une relation linéaire inverse entre les deux paramètres : autrement dit, plus le débit augmente, plus la concentration en glycoprotéines diminue (Mélon *et al.* 1968). La concentration en glycoprotéines peut être évaluée aussi bien par le dosage de la fraction mucopolysaccharidique que par le dosage de la fraction protéinique. Celle-ci mesurée par la réaction au biuret, présente une concentration descendant de 40 à  $5 \text{ mg g}^{-1}$  pour des débits échelonnés entre 1 et  $3 \text{ mg cm}^{-2} \text{ min}^{-1}$ . Au-delà de ce dernier débit la concentration en protéines dont la valeur moyenne est de  $6,5 \text{ mg g}^{-1}$  ne se modifie plus (Fig. 1).

Le présent travail a pour but de présenter les résultats d'une étude comparative de la composition de la sécrétion nasale prélevée chez des sujets atteints de coryza spasmodique d'origine allergique ou d'origine réflexe.

Nous avons recherché s'il existait des critères d'ordre chimique permettant de distinguer l'un de l'autre, les deux types de coryza.

TABLEAU 3 Évolution du débit sécrétoire et de la composition chimique du mucus nasal au cours de la crise allergique (Patient n° 7)

Sensibilisation au pollen de graminées.

Prélevements	Débit sécrétoire (mg cm <sup>-2</sup> min <sup>-1</sup> )	Na ( $\mu$ eq/g)	K ( $\mu$ eq/g)	Ca ( $\mu$ eq/g)	Cl ( $\mu$ eq/g)	Protéines (mg g <sup>-1</sup> )
I. 5 min. après pulvérisation saline téleson	2,08	131,81	12,91	8,68	145,30	23,37
II. 8 min. après pulvérisation antigénique	16,38	116,99	9,80	3,49	122,54	17,47
III. 15 min. après pulvérisation antigénique	21,41	113,23	9,03	2,35	115,32	18,03
IV. 30 min. après pulvérisation antigénique	17,28	116,42	10,85	3,01	118,85	7,59
V. 40 min. après pulvérisation antigénique	17,71	109,48	9,10	3,24	116,03	11,00
VI. 60 min. après pulvérisation antigénique	14,38	113,34	11,67	2,49	110,45	5,30

niveau avant le contact antigénique. Lorsque la concentration initiale est inférieure à 12 mg.g<sup>-1</sup> elle présente une brusque augmentation dès le début de la crise et atteint une valeur 2 à 5 fois supérieure à celle de départ. Cette concentration élevée en protéines ne se maintient que pendant 10 à 20 minutes. Elle diminue dans la majorité des cas et rejoint vers la 60<sup>me</sup> minute, le niveau mesuré chez les sujets normaux si l'on tient compte du débit sécrétoire à ce moment (Tableau 2).

Lorsque la concentration initiale est supérieure à 12 mg.g<sup>-1</sup> elle subit une diminution mais reste néanmoins toujours supérieure aux valeurs normales pendant la plus grande partie de la crise (Tableau 3).

Comme chez les sujets normaux, la fraction mucopolysaccharidique suit une évolution strictement parallèle à la fraction protéinique, ce qui signifie par conséquent que c'est la concentration en glycoprotéines qui a évolué pendant la crise.

B. Au cours de la crise de coryza d'origine réflexe les concentrations en Na, Ca et Cl subissent par rapport aux valeurs normales, une diminution d'autant plus importante que l'hypersecretion est plus abondante. La valeur moyenne de ces différentes concentrations est de 10  $\mu$ eq.g<sup>-1</sup> pour le Na, de 4,78  $\mu$ eq.g<sup>-1</sup> pour le Ca, de 122,83  $\mu$ eq.g<sup>-1</sup> pour le Cl.

La concentration en K ne varie pas de façon significative. La valeur moyenne est de 1,19  $\mu$ eq.g<sup>-1</sup>.

La concentration en protéines est comparable aux valeurs déterminées pour les sujets sains dont le débit sécrétoire dépasse 3 mg.cm<sup>-2</sup> min<sup>-1</sup> (Tableau 4).

TABLEAU 2 *Evolution du débit sécrétoire et de la composition chimique du mucus nasal au cours de la crise allergique (Patient n° 5)*

Sensibilisation au pollen de graminées.

Prélèvements	Débit sécrétoire (mg. cm <sup>-2</sup> min <sup>-1</sup> )	Na (μeq/g)	K (μeq/g)	Ca (μeq/g)	Cl (μeq/g)	Protéines (mg g <sup>-1</sup> )
I Sur muqueuse saine	2,12	131,43	15,92	5,61	147,63	11,80
II 5 min. après pulvéris. sol. saline témoin	2,23	133,18	16,05	5,40	145,21	11,75
III 5 min après pulvéris. sol. antigénique	33,39	118,00	10,43	4,03	123,14	23,84
IV 10 min. après pulvéris. sol. antigénique	30,82	118,42	12,48	4,13	116,18	18,37
V 25 min. après pulvéris. sol. antigénique	20,54	119,32	11,23	4,73	122,06	15,00
VI 60 min après pulvéris. sol. antigénique	17,81	118,88	12,64	4,56	124,63	8,71

muqueuse septale. Pendant la période d'imbibition des papiers collecteurs, le vestibule nasal est obturé à l'aide d'un pince-nez. Le mucus récolté est pesé puis dilué en vue du dosage des éléments inorganiques Na, K, Ca et Cl, ainsi que des protéines.

Chez les sujets du groupe I les prélèvements de sécrétion sont réalisés d'abord au niveau de la muqueuse septale saine, ensuite cinq minutes après la pulvérisation d'une solution témoin ne contenant pas de pollen, enfin, à plusieurs reprises après l'application de l'allergène. Cette application est réalisée en dehors de la période de pollinisation.

Chez les sujets du groupe II la crise est déclenchée par le simple contact du papier collecteur.

## RESULTATS

A Ni le contact du papier collecteur ni la pulvérisation nasale de la solution témoin ne déclenchent chez les sujets du groupe I d'hypersecretion ou de modification quelconque de la composition chimique du mucus.

Par contre, la pulvérisation locale de la solution antigénique spécifique provoque l'apparition d'une violente crise de coryza spasmodique. Au cours de celle-ci, les concentrations en Na, K, Ca et Cl diminuent. La diminution moyenne est évaluée à 13,5 % pour le Na, 33 % pour le K, 43 % pour le Ca et 20 % pour le Cl. Cette diminution des concentrations en éléments inorganiques se maintient pendant toute la durée de la crise (Tableau 2).

La concentration en protéines subit une évolution différente suivant son

(a) La concentration en h est très variable d'un sujet normal à l'autre. Mesurée chez 40 sujets sains, elle oscille entre 12 et 20  $\mu\text{eq}\cdot\text{g}^{-1}$ . Si l'on ne connaît pas le niveau de cette concentration immédiatement avant la crise d'hypersecrétion, on ne pourra considérer comme valeurs anormalement basses et donc typiques d'une étiologie allergique que les concentrations inférieures à 12  $\mu\text{eq}\cdot\text{g}^{-1}$  et comme valeurs élevées typiques d'une étiologie réflexe que les concentrations supérieures à 20  $\mu\text{eq}\cdot\text{g}^{-1}$  dont il faut déduire l'abaissement, évalué à 35% que provoque la réaction anaphylactique — soit 17  $\mu\text{eq}\cdot\text{g}^{-1}$ . Ainsi, il apparaît que la mesure de la seule concentration en h ne permettra de détecter l'étiologie que d'un nombre limité d'hypersecrétions paroxysmiques.

La mesure de la concentration en protéines est un élément de diagnostic plus sûr. En effet des concentrations supérieures à 11 ou 7  $\text{mg}\cdot\text{g}^{-1}$  mesurées au cours des minutes qui suivent le début de la crise doivent être considérées comme témoignant d'une réaction allergique pour autant que l'existence d'une infection locale intercurrente puisse être éliminée.

(b) Le prélèvement de la sécrétion chez un sujet dont le débit sécrétoire est normal ne peut s'effectuer qu'à l'aide d'un matériel hydrophile placé au contact de la muqueuse. Il s'avère donc impossible de connaître la composition du mucus avant la crise, chez des sujets atteints d'hypersecrétion d'origine réflexe puisque le prélèvement déclenche la paroxysme sécrétoire. Il en est de même pour la majorité des allergies nasales chroniques, au cours desquelles se développe progressivement une hypersensibilité tactile de la muqueuse.

D'autre part, au cours des manifestations sécrétoires de ces allergies chroniques, le prélèvement du mucus devra se faire sans attouchement de la muqueuse si l'on veut éviter la production simultanée d'une hypersecrétion réflexe venant modifier la composition chimique du produit de l'hypersecrétion d'origine anaphylactique. Comme nous l'avons montré plus haut, les modifications d'origine réflexe de la composition de la sécrétion consistent notamment en une augmentation de la concentration en h et en une diminution de la concentration en protéines. Aussi, l'irritation tactile de la muqueuse nasale au cours d'une crise d'origine allergique risquera-t-elle de faire perdre à la concentration en h toute valeur diagnostique. Par contre il persistera toujours une élévation de la concentration en protéines témoignant de la réaction anaphylactique.

### CONCLUSIONS

Il existe des différences entre la composition chimique de la sécrétion nasale d'origine allergique et celle de la sécrétion d'origine réflexe. Elles concernent principalement les concentrations en h et en protéines. Au cours des manifestations sécrétoires d'origine anaphylactique la concentration en h diminue tandis que celle en protéines augmente. Au cours des hypersecrétions d'origine réflexe la concentration en h reste élevée comme chez

TABLEAU 4 *Composition du mucus nasal récolté au cours de la crise de cory  $\pi$  spasmodique d'origine réflexe*

Sujets	Age et Sexe	Débit sécrétoire (mg cm <sup>-2</sup> min <sup>-1</sup> )	Na ( $\mu$ eq/g)	K ( $\mu$ eq/g)	Ca ( $\mu$ eq/g)	Cl ( $\mu$ eq/g)	Protéines (mg g <sup>-1</sup> )
1 Pir A	40 a. F	11,67	114,58	17,77	3,33	129,75	6,41
2 Koc M. L.	36 a. F	13,14	101,58	17,60	4,46	118,82	5,77
3 Hex. P	20 a. F	15,87	116,89	18,81	6,81	132,52	4,39
4 Rig. L.	21 a. F	1,00	104,36	20,37	6,30	128,10	5,12
5 Pir J	6 a. F	18,00	11,46	16,28	3,65	130,64	7,31
6 Méd. C.	23 a. F	18,33	113,57	18,56	4,83	121,80	6,31
7 Dem J. P.	27 a. M	18,81	109,12	15,10	2,90	120,36	7,22
8 Con J	34 a. F	10,20	95,78	1,75	2,68	108,99	5,39
9 Ama. P	21 a. F	24,00	93,83	16,45	4,83	121,32	5,66
10 Cho. J	26 a. F	25,70	104,02	16,14	3,30	114,25	6,70
Valeurs moyennes		18,31	107,15	17,19	4,29	122,63	6,02
Valeurs moyennes normales		1 à 3	127,0	1,30	4,88	138,82	5 (pour les débits > à 3 mg)

## DISCUSSION

Que l'origine de l'hypersécrétion soit allergique ou réflexe, les concentrations en Na, Ca et Cl évoluent dans le même sens : toutes trois diminuent. Les concentrations en Na paraissent plus basses dans les cas d'hypersécrétion  $\pi$  origine réflexe tandis que les concentrations en Ca et en Cl semblent diminuer davantage dans les cas d'hypersécrétion de nature anaphylactique. Mais nos recherches n'ont pas porté sur un nombre suffisant de patients pour que des limites de concentrations correspondant à l'un ou l'autre type de coryza puissent être établies.

Par contre deux composants du mucus — le K et la fraction protéinique — présentent une évolution divergente suivant que l'hypersécrétion est d'origine allergique ou réflexe. Dans la première éventualité la concentration en K diminue, celle en protéines augmente. Dans la seconde éventualité, la concentration en K garde une valeur très élevée comparable à celle mesurée chez le sujet normal tandis que la concentration en protéines présente des valeurs très basses.

Sur le plan pratique, les valeurs de concentration en K et en protéines mesurées chez un patient atteint d'hypersécrétion paroxystique peuvent-elles être utilisées en vue d'un diagnostic étiologique? Pour répondre à cette question, il faut prendre en considération, d'une part, les niveaux significatifs de concentration des deux composants du mucus et, d'autre part, la méthode de prélèvement de la sécrétion.

## PERMEABILITY OF THE INNER EAR MEMBRANES

CH. V. ILBERG and K. H. VÖRTEKX

*From the Otolaryngological Clinic University of Frankfurt/Main Germany*

Soluted substances can penetrate through epithelial layers either by free diffusion through the intercellular spaces or by transcellular transport. By injection of *Ih*, as a tracer into peri and end lymph we found a typical transcellular transport of the tracer through Reissner's membrane in micropinocytosis vesicles. Contrary to this observation, the tracer entered the "cortilymph spaces" from sc. tympani by free diffusion through open intercellular spaces. The sensory cells take up the tracer at their infranuclear area. We therefore conclude that cortilymph is perilymph and the hair cells of Corti's organ are mainly supplied from the perilymph side.

We know two ways in which soluted substances can penetrate an epithelial layer either through the cells or between their intercellular spaces. Since the investigations of Farquhar & Palade (1965) we assume that there is no free passage through intercellular spaces closed by a "Zonula occludens". Apart from other mechanisms without morphological characteristics, soluted substances can be transported through epithelial cells by membrane vesiculation. This mechanism we call "pinocytosis" or rather cytopempsis (Moore & Ruska, 1957). Several authors have described similar observations at different parts of the cochlear.

To study further details of ion and fluid transport between the endolymphatic and perilymphatic spaces we injected a colloidal solution of thorium dioxide (*Ih*) as a tracer into the endo- and perilymph of the guinea pig. Because of their small size (40 Å) these electrondense particles are suitable as a tracer for cochlear study by electronmicroscopy. We present our findings at Reissner's membrane and the organ of Corti using this method.

Five minutes after injection of the tracer into the perilymph we found that the intercellular spaces (ICS) between the connective cells of Reissner's membrane on the scala vestibuli side were filled with *Ih*, as well as the basement membrane. Furthermore, *Ih* penetrated into the ICS between the epithelial cells, passed through the desmosomes, and was finally stopped by the Zonula occludens. We never saw the tracer within the Zonula occludens or desmosomes themselves. At the basal and junctional surfaces of the epithelial cells we found micropinocytosis vesicles (MPV) crossing their cytoplasm to the endolymphatic surface within 30-60 min. Sometimes we observed MPV pouring out their contents into the endolymph.

le sujet normal tandis que celle en protéines est très basse. Ces différences peuvent servir de critères diagnostiques permettant de distinguer les deux types d'hypersécrétion. Ne sont cependant significatives que les concentrations en K inférieures à  $12 \mu\text{eq g}^{-1}$  ou supérieures à  $17 \mu\text{eq g}^{-1}$  mesurées dans des échantillons de mucus prélevés en l'absence de toute irritation de la muqueuse. Par contre, en l'absence d'infection locale, toute élévation de la concentration en protéines au-dessus d'une valeur de 5 à  $7 \text{ mg g}^{-1}$  témoigne d'une réaction allergique qu'il y ait ou non des manifestations réflexes concomitantes.

### ZUSAMMENFASSUNG

Es bestehen Unterschiede zwischen der chemischen Zusammensetzung der Nasensekrete allergischen Ursprunges und der Sekrete reflektorischen Ursprunges. Dieses betrifft hauptsächlich die Konzentration an K und an Proteinen. Im Laufe der Absonderungsvorgänge (= Sekretion) anaphylaktischen Ursprunges wird die Konzentration an K kleiner während diejenige an Proteinen größer wird.

Im Laufe der übergroßen Absonderung (= Hypersekretion) reflektorischen Ursprunges bleibt die Konzentration an K sehr hoch so wie beim normalen Menschen dagegen die an Proteinen sehr klein.

Diese Unterschiede können als Diagnose Erkennungszeichen dazu dienen, die zwei Arten Hypersekretionen zu unterscheiden. Doch die Konzentrationen an K sind nur von Wichtigkeit wenn sie tiefer liegen als  $12 \mu\text{eq g}^{-1}$  oder höher als  $17 \mu\text{eq g}^{-1}$  und wenn die Schleimproben in Abwesenheit jeglicher Entzündung der Schleimhaut gemacht wurden. In Abwesenheit jeglicher örtlichen Entzündung zeigt dagegen jede Erhöhung der Konzentration an Proteinen über 5 bis  $7 \text{ mg g}^{-1}$  eine allergische Reaktion an, ganz gleich ob begleitende reflektorische Äußerungen bestehen oder nicht.

### SUMMARY

There are differences between the chemical composition of nasal secretion of allergic origin and that of reflex origin: they mainly concern K and protein concentrations. During secretory manifestations of anaphylactic origin K concentration decreases, while that of protein increases. In hypersecretions of reflex origin, K concentration remains high as in normal individuals, while that of proteins is very low. These differences may serve as diagnostic criteria for distinguishing both types of hypersecretion. However only those K concentrations lower than  $12 \mu\text{eq g}^{-1}$  or higher than  $17 \mu\text{eq g}^{-1}$  measured on mucous samples taken from mucosa devoid of any irritation have any significance. On the contrary, when there is no local infection any rise in protein concentration higher than 5 to  $7 \text{ mg g}^{-1}$  evidences an allergic reaction whether or not there are any concomitant reflex manifestations.

### BIBLIOGRAPHIE

- MELCHIOR, R. 1930. Allergie et troubles secrets de la muqueuse nasale. *Acta oto-laryng. Belg.* 4, 8.  
 MÉLOX, J. et SCHOFFELEERS, E., 1966. La composition chimique de la sécrétion nasale normale. *Rhin. logi. International* 4, 71.  
 MÉLOX, J., LEDOUX, A. et SCHOFFELEERS, E., 1968. Mucine content of nasal secretion. *Sou. presse*.

Clinique O. R. L., Hôpital d'Anvers  
 Liège, Belgique

ever there is no free communication between endo- and perilymph through the ICS of the epithelial cells as they are sealed by impermeable terminal bars. Thus an active transport through the epithelial cells by means of pinocytosis and/or cytopempsis is involved.

In the organ of Corti and its supporting structures we have a completely different situation. Between perilymph on one side and endolymph on the other there is a third fluid space which was called the "cortilymph space" by Engström (1960). Several electrophysiological and biochemical investigations suggested that cortilymph and perilymph should have the same composition. However no connection has been demonstrated so far between the two fluids by electronmicroscopy. After the injection of *th* into the endolymph we saw the tightly packed particles on the endolymphatic surface of the sensory cells and the reticular membrane. The intervening intercellular spaces, sealed by marked Zonula occludens, could not be passed by the *th* particles. There was no uptake of *th* to be seen in this region. Contrary to this finding, the tracer injected into the scala tympani passed after only a few minutes through the tympanic covering-layer and the fibers of the basilar membrane. Thirty to sixty minutes later the particles reached the cortilymph spaces through the ICS. We saw the tracer particles between the Deiters cells surrounding the outer spiral fibers between the pillar cell and the unmyelinated nerve fibers at the habenula perforata, so that the particles were lining the surface of the cortilymph spaces. Further the labyrinth of the ICS around and below the inner hair cells contained *th* particles ascending up to the terminal bars at their hair bearing surface.

Even the synaptic cleft between the inner and outer hair cells and their nerve endings contained *th* particles. We demonstrated an uptake of the tracer into the sensory cells at their infranuclear surface (Fig. 1).

With our method we have shown that the sc. tympani and the tunnel spaces communicate freely through open ICS but are closed against the subecl. endolymph. We therefore have proved that the so-called "cortilymph spaces" contain perilymph. This means that the outer and inner hair cells of the organ of Corti will be mainly if not completely supplied with nutritious substances from the perilymph. We assume that the oxygen supply of the sensory cells occurs from the perilymphatic side too.

Referring to our diagram, shown at the beginning, of two different parts of the cochlea we could demonstrate that both transcellular transport as well as intercellular diffusion play an important role in the inner ear fluid balance.

## RESUME

La injection d' thoriumdioxid dans la perilymphe nous a permis d'étudier les structures membranaires de l'oreille interne par rapport à leurs caractéristiques de perméabilité et de résorption. La scala media fonctionnait comme





FIG. 1 Infranuclear region of an inner hair cell 60 min after injection of thorium dioxide into the perilymph. The trace can be seen in the synaptic vesicles (Syc) between the inner hair cell (IHC) and a myelinated nerve ending (NE). Many micropinocytosis vesicles take up the tracer into the hair cell and even into the nerve cell (arrows).

After injection into the scala media the same mechanism in the opposite direction was to be seen. The tracer could not pass through the Zonula occludens of the ICS from this side either. Numerous tracer-filled MPV invaginated into the epithelial cells from the endolymphatic surface, travelling through their cytoplasm towards the perilymph. We often saw MPV-containing tracers entering the typical cisternae of Reissner's membrane thus forming a sort of "multivesiculated body".

By this method Reissner's membrane was proved to be permeable for fluid transport from either side. The layer of connective-like cells and the basement membrane allowed a free passage even for macromolecules. How

## STREPTOMYCINSPIEGEL IN DER PERILYMPHE DES MENSCHEN

A. MEYER zum GOTTENBERG und H. F. STUPP

Düsseldorf, Deutschland

In früheren Untersuchungen wurde durch Stupp im Tierversuch am Meerschweinchen gezeigt, daß die Aminoglykosidantibiotika (Streptomycin, Kanamycin) in der Peri- und Endolymphe sehr hohe Konzentrationen und sehr lange Verweildauer erzielen. Der gleiche Effekt wird nunmehr auch in der Perilymphe des Menschen für parenteral injiziertes Streptomycin in therapeutischer Dosierung nachgewiesen.

Das Schrifttum über die ototoxischen Wirkungen der Aminoglykosidantibiotika ist fast unüberschaubar geworden. Verhältnismäßig wenige Untersuchungen beschäftigen sich jedoch mit den pharmakokinetischen Aspekten dieser Frage. Wir sind der Überzeugung, daß dieser Aspekt nicht nur mit den bereits vorliegenden Tierexperimentellen Befunden sehr interessante Ergebnisse in der speziellen Frage der Ototoxikose erbracht hat, sondern darüber hinaus geeignet ist, zu allgemeinen physikochemischen Fragen der Innenohrbilogie einen Beitrag zu leisten.

Pharmakologische Wirkungen und toxische Nebenwirkungen können nur zustande kommen, wenn das Pharmakon in ausreichender Menge bzw. Konzentration mit den reagierenden Bestandteilen der Zelle in Kontakt kommt. Blut bzw. Plasmaspiegel einer Substanz sind keineswegs ohne weiteres für die Konzentration der Substanz am Wirkungsort repräsentativ. Für die sehr unterschiedliche Verteilung der Substanzen im Organismus und für ihren Verbleib sind Resorption, Distribution und Elimination entscheidend.

Für derartige pharmakokinetische Untersuchungen an der Innenohrlymphe sind Natrium- und Kaliumionen, Glukose usw. nicht so sehr geeignet, wie es auf den ersten Blick erscheinen mag. Die Vielseitigkeit ihres ubiquitären Vorkommens, ihrer Stoffwechselaktivität und ihres Transportes im Organismus macht die Beurteilung sehr schwierig.

Für Indikatoren Substanzen, die sich durch definiertere und überschaubare Eigenschaften auszeichnen, geeigneter wie z. B. Inulin, das bei Clearance Untersuchungen der Niere Anwendung findet. Das chemisch indifferente und nicht resorbierbare Inulin, das nicht in die Zelle eindringen und infolgedessen auch nicht sezerniert werden kann, kann nur durch Filtration oder Diffusion transportiert werden. Inulin gelangt nach Untersuchungen von Stupp zwar ohne Schwierigkeiten in die Perilymphe, aber sehr schwer wieder heraus. Die verzögerte Ausscheidung ist eine Folge unzureichender Rückresorption. Diese „Inulinclearance“ des Innenohres ergibt nun later

An Hr. *Wersäll* Einen toxischen Effekt des Th. sahen wir bei unseren Versuchen noch nicht. Es stellt sich möglicherweise nach längeren Inkubationszeiten erst ein. Die Pinocytose des Th. aus dem synapt. Spalt konnten wir nach 60 min bis etwa in kernnähe der Sinneszellen (Golgi Zonen?) verfolgen. An der Stria vasc. findet eine deutliche Resorption des Th. aus der Endolymph statt.

An Hr. *Meyer am Gottesberge* Eine freie Diffusion durch Reissner Membran halten wir aufgrund unserer Beobachtungen für sehr unwahrscheinlich. Möglicherweise wird Inulin transzellulär transportiert.

An Hr. *Engström* Nachdem Makromoleküle bis 40 Å Grösse von der sc. tympani in die „Corti Lymphräume“ gelangen können ist kaum anzunehmen, dass ein Unterschied zwischen Inhalt der Cortilymphräume und der Perilymphe besteht.

To Mr. *Palva* By our method we got no information on any fluid movement, neither perilymph nor endolymph.

To Mr. *Spoendlin* Yes, we sometimes see terminal bars at the basilar membrane. But they seem not to close the whole length of the ica, so that the tracer can pass them. We too are irritated by the very few particles in the tunnel space. But they penetrate very slowly. After a long period there might be more of them. However small amounts of additional active transport into this region cannot be excluded. Limbus spiralis apart from epithelial cells, the whole space is filled with perilymph. Interdental cells take up great amounts of the Th. by pinocytosis (slide).

à Mr. *Portmann* En effet le scala media est un espace totalement fermé par son epithelium et il n'y a pas d'échange entre péri- et endolymph par diffusion. Par notre méthode il est impossible de dire quelque chose sur le mouvement de l'endolymph et l'endroit de sa production.

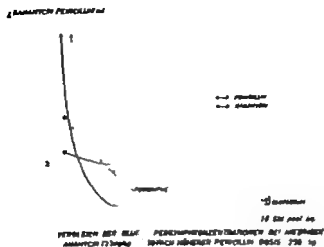


Abb. 2. Vergleich der Blut- d. Perilymphkonzentrationen bei niedriger Kanamycin-  
25 mg/kg d. 10fach höherer Penicillin-Dosis (250 mg/kg)

Polymyxine welche neurotoxisch, aber nicht ototoxisch sind, gehen gleichfalls nicht in die Innenohrymbe über

Zur Behandlung entzündlicher Innenohrerkrankungen sollten daher nur solche Antibiotika herangezogen werden, die am Wirkungsort eine hohe Konzentration erreichen. Das sind die ototoxischen Antibiotika. Jedoch muß bei entzündlichen Innenohrerkrankungen die Dosierung der ototoxischen Antibiotika niedrig gehalten werden, da nach den Untersuchungen von Voldrich & Vabreck unter entzündlichen Bedingungen im Innenohr besonders hohe Konzentrationen dieser Antibiotika auftreten.

Von theoretischem und klinischem Interesse ist nun die Frage, ob sich die erwähnten, im Tierversiment am Meerschweinchen erhobenen Befunde auch beim Menschen bestätigen lassen. Ergänzend zu den tierexperimentellen Befunden wurden daher analoge Untersuchungen am Menschen durchgeführt, die uns die Frage beantworten sollten, ob beim Menschen ebenfalls eine peristische Kumulation der toxischen Antibiotika vorkommt, die für die Otitiden ursächlich verantwortlich gemacht werden kann.

Die Untersuchungen wurden an insges. 26 Otosklerosepatienten vorgenommen, bei denen die Gelegenheit gegeben war anlässlich einer Hörer-

Standards post 1911	Thio- cyanide	Peri- cyanide	Serum	Heart	Liver	Gastric	
5	10.5	20.5	10.01	0.81	0.6	0.102	7 ml. brw. s.
1	8.5	8.8	1.17	0.15	0.16	0.018	7 ml. brw. s.

Tab. 2. Vergleich des Kanamycin gehaltes verschiedener Organe (234 mg Kanamycin kg)

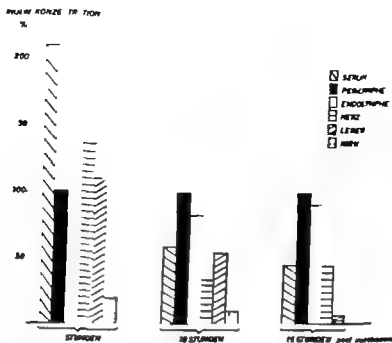


Abb. 1 Vergleich der Inulinkonzentration in Serum, Innenohrlymph und Organen, bezogen auf Inulinkonzentration der P. Lymphe = 100

essante Parallelen zu dem Verhalten der Antibiotica der Obligosaccharidgruppe, die als Struktureinheit ebenfalls einen Zucker aufweisen (Streptomycin Kanamycin Neomycin Framycetin Gentamycin und einige weniger bekannte Verbindungen). Diese Zuckerantibiotica sind ebenfalls nicht resorbierbar und können nicht sezerniert werden als Substanzen des extrazellulären Raumes können sie nur durch Diffusion und Filtration in die Innenohrflüssigkeiten gelangen. Bei gleicher d. h. äquimolarer Dosierung und im niedrigen, toxisch nicht wirksamen Dosissbereich verhält sich Kanamycin sehr ähnlich wie Inulin. Seine Konzentration in der Innenohrlymphe ist außerordentlich hoch und übertrifft diejenige anderer Organe und die des Blut bzw. Serumspiegels um ein Vielfaches. Seine Verweildauer ist ungewöhnlich lang (Stupp).

Noch deutlicher kommt dieser Kumulationsprozeß bei der für tierexperimentelle Untersuchungen üblichen ototoxischen Dosierung von 250 mg pro kg zum Ausdruck.

Die Höhe der Konzentration und die Dauer der Einwirkungszeit im Innenohr sind u. E. für die spezifisch ototoxische Wirkung von ausschlaggebender Bedeutung.

Im Gegensatz zu dem Verhalten der Zuckerantibiotica liegt die Konzentration des Penicillins stets deutlich unter der des Blutspiegels, Tetracycline erreichen überhaupt keinen wirksamen Innenohrspiegel, auch wenn sie in allen anderen Organen in hoher Konzentration nachweisbar sind. Tetracycline können bekanntlich die Blutliquorschranke nicht überschreiten. Das Gleiche gilt offenbar für die Blut-Innenohr-Lymphschranke (Stupp).

nen, die aber im Vergleich mit den sonst in anderen Organen, wie Herz, Leber oder Gehirn vorliegenden Konzentrationen, wie sie vorhin dargestellt waren doch noch relativ hoch sind.

Bemerkenswert ist, daß der Streptomycinsulfbloodspiegel zwischen 2 und 5 Stunden nach der Infektion nur wenig, und zwar von 24  $\gamma$ /ml auf 20  $\gamma$ /ml abnimmt, während die Perilymphkonzentration von 10  $\gamma$ /ml auf über 20  $\gamma$ /ml ansteigt. Würde man noch länger 12 oder 24 Stunden warten, so käme diese Tendenz, eine Abnahme des Blutspiegels bei zunehmender oder gleichbleibender Innenohrkonzentration, noch deutlicher zum Ausdruck. Derartige Untersuchungen werden zur Zeit durchgeführt.

Auffällig sind ferner bei diesen Versuchen am Menschen die ungewöhnlich großen Schwankungen der Perilymphkonzentrationen bei den einzelnen Versuchspersonen, die eine statistische Auswertung sehr erschweren. Die Blutspiegel waren dagegen weitgehend konstant, so daß eine renale Ausscheidungslörung als Ursache ausgeschlossen werden kann. Trotz niedriger Dosierung überraschten in einigen Fällen ausserordentlich hohe Perilymphspiegel, die die Blutkonzentration um ein Vielfaches übertrafen. Man muß sich fragen, ob die bei einigen Menschen vorkommenden extrem hohen Streptomycinkonzentrationen im Innenohr für die in der Klinik gelegentlich bei kleiner Dosis völlig unerwartet auftretenden und daher sehr gefürchteten Innenohrschädigungen verantwortlich gemacht werden können. Damit könnte möglicherweise ein Phänomen erklärt werden, für das wir bisher nur die Bezeichnung individuelle Überempfindlichkeit hatten.

### SUMMARY

In ex luv xam alimx on guinea pigs it was shown by M pp that th aminoglycosidantibiotics (streptomycin, kanamycin, neomycin) obtain a very high concentration and a very long duration in the perilymph and endolymph. The same effect has now been proved in the perilymph of men with streptomycin injected in a therapeutic dose.

### RESUME

Stupp a déjà démontré par des expériences faites sur des cobayes que les aminoglycosides antibiotiques obtiennent une concentration très élevée et de longue durée dans la périlymphe et dans l'endolymphe. Le même effet est maintenant aussi révélé dans la périlymphe de l'homme en cas d'une injection streptomycine dans une dose thérapeutique.

Dr med. A. Mey am Göttinger  
Moorstrasse 3,  
Düsseldorf, Deutschland

### DISCUSSION

I find that we have referred to the paradox phenomena observed in experimental animal and in human cathectes. The lower dry causing greater damage in



Abb. 4 Streptomycinsulfatkonzentrationen in Serum und Perilymphe des Menschen bei niedriger Dosis von 20 mg/kg

bessernden Operation (Stapedektomie) Perilymphe zu entnehmen. In zahlreichen anderen Fällen scheiterte die Perilymphabnahme beim Menschen meistens infolge zu starker Blutungen. Diese Versuche wurden von vornherein verworfen. Für diese Untersuchungen wurden nur einwandfreie Perilymphe ohne Blutbeimengungen benutzt.

Die Patienten erhielten eine einmalige Injektion von Streptomycinsulfat in einer normalen therapeutischen Dosierung von 20 mg/kg und zwar eine Gruppe 2 Stunden vor der Perilymphabnahme und eine andere Gruppe 5 Stunden vor der Entnahme.

Die Lymphe wurde nach vorheriger sorgfältiger Blutstillung mit Hilfe feinsten selbstgefertigter Glaskapillaren aus einer kleinen Perforation in der Steigbügel Fußplatte entnommen. Gleichzeitig wurde Venenblut abgenommen, das Serum abzentrifugiert und die Proben Serum und Perilymphe bis zur Durchführung der bakteriologischen Untersuchung eingefroren.

Die Bestimmung des Antibiotiegehaltes erfolgte auf bakteriologischem Wege durch Messung der antibiotischen Aktivität. Es wurde das allgemein in der Bakteriologie übliche Agar Diffusions-Verfahren angewendet und zwar bei Serum und Gewebe in Form des Loch Testes und bei Perilymphe mit Hilfe des Blattchen Testes. Verwendet wurden Filterblattchen mit einem Durchmesser von 5 mm. Als Teststamm diente *B. subtilis* ATCC 6633. Die niedrigste feststellbare Streptomycinkonzentration betrug im Serum 0,01  $\gamma$ /ml bzw. 0,08  $\gamma$ /g in der Perilymphe dagegen 0,5  $\gamma$ /ml.

Während bei den tierexperimentellen Untersuchungen in der Regel höhere Dosen und vor allem toxischere Antibiotika wie Kanamycin verwendet wurden, mußten wir uns bei den jetzigen Versuchen am Menschen um jegliches Risiko zu vermeiden, auf möglichst niedrige Dosen und das am wenigsten ototoxische Antibiotikum dieser Gruppe, das Streptomycinsulfat, beschränken. In früheren Arbeiten hatten wir schon eingehend zeigen können, daß die Kumulation bei höheren toxischen Dosierungen und vor allem bei den toxischeren Substanzen wie Neomycin weitaus am ausgeprägtesten ist.

Erwartungsgemäß zeigen die Streptomycinsulfat Spiegel in der Perilymphe im therapeutischen Bereich beim Menschen nur mäßig hohe Konzentrationen.

## REGIONAL DIFFERENCES IN SENSITIVITY OF THE VESTIBULAR SENSORY EPITHELIA TO OTOTOXIC ANTIBIOTICS

H. H. LINDEMAN

*From the Otolaryngologic Department of the University of Gothenburg  
Gothenburg Sweden*

The effect of intratympanic application of streptomycin and parenteral injection of kanamycin upon the vestibular sensory epithelia have been studied with the surf-co specimen technique. Clear regional differences in vulnerability to these antibiotics have been noticed. The sensory epithelia of the cristae ampullares were more vulnerable than the macula utriculi, which in turn was distinctly more vulnerable than the macula sacculi. The degenerative changes involved primarily the central part of the crista epithellum and the striola of the maculae.

Antibiotics of the streptomycin group have side effects, one of the most serious of which is a toxic action upon the mechanisms of hearing and equilibrium. Since Berg (1949) and Courvé (1949) first observed degenerative changes in the vestibular sensory epithelia and in the organ of Corti of streptomycin-treated animals, there has been general acceptance of the inner ear sensory epithelia as the primary sites of these toxic effects.

Recent studies have revealed a specific pattern of degeneration in the organ of Corti following administration of ototoxic antibiotics (Beck & Krabl 1962, Hawkins & Engström, 1964, Kohonen, 1965). Differences in sensitivity to streptomycin among the cristae and the maculae and even within each crista, have been described by some authors; however the results are to some extent controversial.

By the aid of light and phase contrast microscopic studies of surface preparations from the vestibular sensory regions, clear regional differences in structure have been demonstrated (Lindeman, 1969). Against this background the present study was carried out in order to analyze the overall pattern of damage in the vestibular sensory epithelia following administration of ototoxic antibiotics. A short communication of some of the present findings has been published (Lindeman, 1966).

### MATERIAL AND METHODS

The subjects of the experiment were 20 guinea pigs (non-albinos) of 250-350 g body weight and having a normal pinna reflex. The animals were divided into two groups.

This work was supported by the Norwegian Research Council for Science and the Humanities and by the Physiological Psychology Branch, Office of Naval Research, Washington D.C. under Contract N 62359 4261 with H. Engström.



individual animals, and vice versa. Regenerative or reparative fissures play an important role in the end-result.

*F. Escher* I would like to mention the experiments on monkeys in the Berne clinic by M. Neiger. It could be proved that the concentration of streptomycin in the perilymph is rising during 24 hours and retained inside as in a prison. This is also a confirmation of the results of Mr Meyer & Gottesberge.

*A. Meyer und Gottesberge* (Antwort) Unsere Untersuchungen erklären sicherlich nicht alles und nicht die Vorgänge in den Zellen. Aber wie Paracelsus sagt, liegt der Unterschied ob ein Ding ein Gift sei oder nicht in der Dosis, d. h. in der Konzentration.

## REGIONAL DIFFERENCES IN SENSITIVITY OF THE VESTIBULAR SENSORY EPITHELIA TO OTOTOXIC ANTIBIOTICS

H. H. LINDEMAN

*From the Otolaryngological Department of the University of Gothenburg  
Gothenburg Sweden*

The effect of intratympanic application of streptomycin and parenteral injection of kanamycin upon the vestibular sensory epithelia have been studied with the surface specimen technique. Clear regional differences in vulnerability to these ototoxic antibiotics have been noticed. The sensory epithelia of the cristae ampullares were more vulnerable than the macula utriculi which in turn was distinctly more vulnerable than the macula sacculi. The degenerative changes involved primarily the central part of the crista epithelium and the striae of the maculae.

Antibiotics of the streptomycin group have side effects, one of the most serious of which is a toxic action upon the mechanisms of hearing and equilibrium. Since Berg (1949) and Coussé (1949) first observed degenerative changes in the vestibular sensory epithelia and in the organ of Corti of streptomycin-treated animals, there has been general acceptance of the inner ear sensory epithelia as the primary sites of these toxic effects.

Recent studies have revealed a specific pattern of degeneration in the organ of Corti following administration of ototoxic antibiotics (Beck & Kralj, 1966; Hawkins & Engström, 1964; Kobonen, 1965). Differences in sensitivity to streptomycin among the cristae and the maculae and even within each crista, have been described by some authors; however the results are to some extent controversial.

By the aid of light and phase contrast microscopic studies of surface preparation from the vestibular sensory regions, clear regional differences in structure have been demonstrated (Lindeman, 1969). Against this background the present study was carried out in order to analyze the overall pattern of damage in the vestibular sensory epithelia following administration of ototoxic antibiotics. A short communication of some of the present findings has been published (Lindeman, 1967).

### MATERIAL AND METHODS

The subjects of the experiments were 20 guinea pigs (non-albinos) of 250-300 g body weight, and having a normal pinna reflex. The animals were divided into two groups.

This work was supported by the Norwegian Research Council for Science and the Humanities and by the Physiological Psychology Branch, Office of Naval Research, Washington D.C. under Contract N 00019-62-0-4264 with H. Engström.

1 In 15 animals a varying dose (5–20 mg) of streptomycin sulphate, dissolved in 0.1 ml Ringer fluid was injected through the tympanic membrane into the middle ear of one side under mebumal anaesthesia. The other ear served as control. Four–five hours after the injection, the animals showed a falling tendency towards the operated side, spontaneous nystagmus towards the opposite side, and ataxia. The pinna reflex disappeared. The vestibular symptoms were considerably reduced after a few days, but during the whole period of observation the animals showed ataxia. The animals were sacrificed 2–8 days after the injection.

2 Five animals were treated by parenteral injections of kanamycin sulphate 300 mg/kg daily over a period of 8–15 days. None of the animals revealed any apparent vestibular symptoms. The animals were sacrificed 5–15 days after completing the treatment.

The temporal bones of the guinea pigs were fixed/stained in 1.0% osmic acid solution or fixed in a solution of equal parts of methanol and ether and stained with Giemsa solution. Five temporal bones were embedded in epon and sectioned for phase contrast microscopy. In the other temporal bones, the vestibular sensory epithelia were dissected free for light and phase contrast microscope studies with a technique described by Lindeman (1969). The organ of Corti was studied using the surface specimen technique (Engström *et al.* 1968).

## OBSERVATIONS

After both streptomycin and kanamycin administration, degenerative changes could be observed in the inner ear. In the vestibular part these degenerative changes were restricted almost completely to the sensory cells. Changes in the sensory cell nuclei were usually the first signs of damage to the epithelium, as studied in light microscopy (Fig. 1). In some specimens a moderate swelling of the nuclei could be observed, the nuclei appearing larger and fainter than normally. More frequently agglomeration and displacement of the chromatin towards the periphery of the nucleus of the sensory cell could be seen. Furthermore, pyknotic and fragmented nuclei, staining strongly with Giemsa, were observed. Before disintegration of the nuclei, a disarrangement of the sensory hairs was seen. The hairs spread out on the surface of the epithelium and often clumped together (Fig. 2). Protrusions of the cytoplasm of the sensory cells into the endolymphatic space, occasionally seen in normal animals, occurred frequently in the experimental animals. A final stage was the so called "collapse figure" indicating a complete degeneration of the sensory cell (Fig. 1). The collapse figures were easily identified and permitted exact quantitative estimates of sensory cell loss. After a few weeks, however, the supporting cells surrounding the degenerated sensory cell filled up the gap of the previous sensory cell, and the collapse figure disappeared. This was a stage comparable with a "phalangeal scar" in the organ of Corti (see Engström *et al.* 1968). In

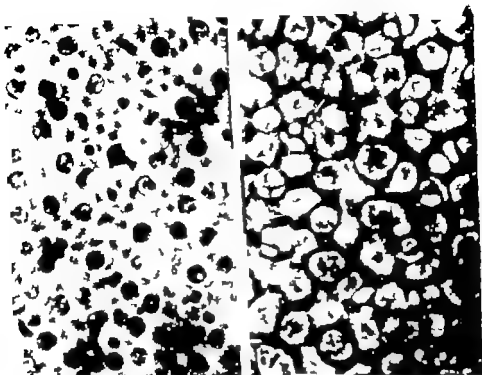


FIG. 1. Surface preparations of the vestibular sensory epithell of the guinea pig after administration of streptomycin. (a) Macula (tricle) showing nuclei of both normal and degenerating sensory cells. Clumping of the chromatin, pyknosis, and fragmentation of the nuclei illustrated different degenerative stages. Glemsa stain (mag. 757). (b) Sensory epithellum of crista ampullares revealing partial loss of sensory cells. The collapse figure (arrow) indicates complete degeneration of a sensory cell. Orcein acid fast (mag. 1575).

contrast, whereas in the organ of Corti the position of a lost sensory cell may be easily observed due to the regular cellular pattern, it was more difficult to localize the former position of a sensory cell in the vestibular sensory epithell, as the cellular pattern in these regions is normally rather irregular (Lindeman, 1960).

Other changes could also be observed in the degenerated vestibular sensory epithellum. After a loss of about 50% of the sensory cells, a reduction in height of the epithellum was noticed. In addition, an increased amount of osmophilic granules could often be seen.

#### *A. Degeneration pattern following application of streptomycin into the middle ear*

The control ears were all normal. Degenerative changes in the vestibular apparatus as well as in the organ of Corti were noticed in all the operated ears. The extent of these changes could be related to a certain degree to the amount of streptomycin injected. Animals treated with large doses of strep-

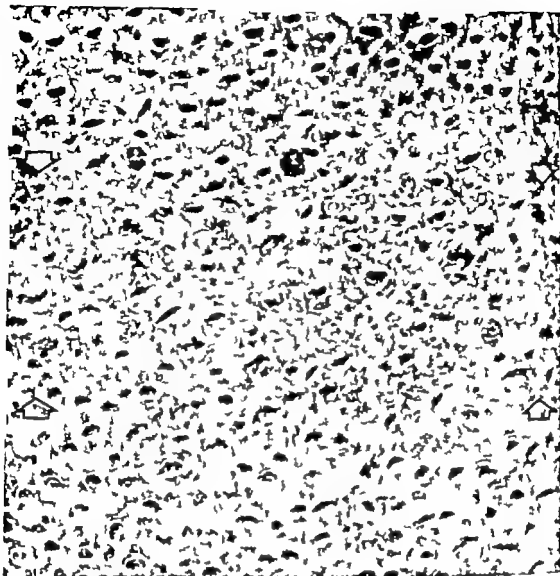


FIG. 2. Surface preparation from macula utriculi of a guinea pig exposed to kanamycin. The hair bundles of the sensory cells are seen in the striola (between arrows); most of the hair bundles are lost or appear different from normal. Osmic acid fixation. 96.

tomycin showed early and extensive degenerative changes. Thus, only 2 1/2 days after treatment with massive doses of streptomycin a considerable loss of vestibular and cochlear sensory cells could be noticed. Animals treated with smaller doses showed less pronounced morphological changes in most cases, and these developed more slowly.

The degeneration of the vestibular sensory epithelia followed a specific pattern. The sensory epithelia of the crista ampullares were more vulnerable than the macula utriculi, which in turn was clearly more vulnerable than the macula sacculi. Furthermore, distinct regional differences in sensitivity were apparent within each sensory region.

On both the macula sacculi and the macula utriculi, the degenerative changes involved the striola primarily. Only when the loss of sensory cells



FIG. 2. Guinea macula utricle (a) Fig. 2. The microscope is focused on the epithelial surface of the utricle. Some collapse figures are seen. Arrow indicates direction of morphological polarization of sensory cells, where basal bodies may be identified. Direction of polarization is reversed in the middle of the striola (544).

In this region as extensive were degenerative changes observed in the periphery of the epithelium. On the cristae the central regions of the sensory epithelium were more vulnerable than the periphery. All three cristae showed about the same extent and pattern of degeneration.

As seen in light microscopy the supporting cells of the vestibular sensory epithelium appeared normal, as did the cupulae and the statoconium membranes.

In addition to vestibular changes, damage to the organ of Corti was observed. The basal coils of many cochleas showed a complete loss of outer hair cells, and often a degeneration of Deiters cells and pillar cells as well. The degeneration of outer hair cells gradually decreased towards the apex, whereas an increasing number of degenerated inner hair cells were observed. The outer hair cells showed a gradient of decreasing vulnerability from the first to the third row.

#### II. Degeneration pattern following parenteral injections of kanamycin

Four out of five animals in this group revealed degenerations of the inner ear sensory cells. In two animals the macula sacculi appeared grossly normal, the only degenerative changes being found in the striola of the macula utriculi and in the central region of the crista (Figs. 2, 3 and 4). Two animals showed slight loss of sensory cells in the striola of the macula

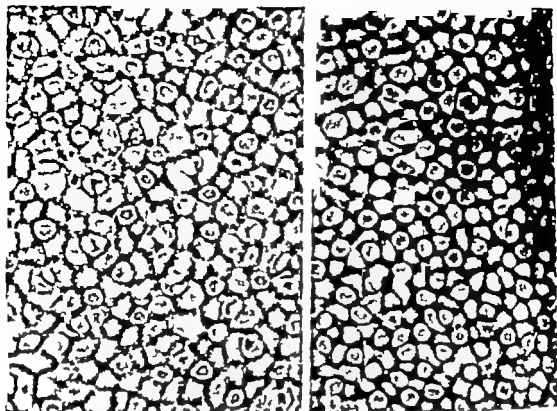


FIG. 4 Two photomicrographs from the same crista ampullaris of guinea pig treated with kanamycin. (a) In the central area a high percentage of the sensory cells are degenerated and appear as collapsed figures (arrows) (b) In the periphery the sensory epithelium looks completely normal, with the exception of one single degenerated cell (arrow) a and b 784

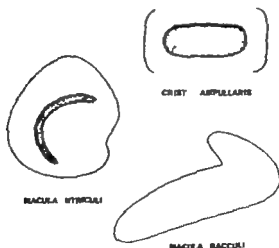


FIG. 5 Diagram illustrating the regional differences in vulnerability of the vestibular system. The density of cells indicates the relative sensitivity of the sensory cells in the different areas. The sensory epithelium of the crista is more vulnerable than the macula utriculi, which in turn is less vulnerable than the macula sacculi. The central areas of the crista and the utricle of the macula are more sensitive than the peripheral parts.

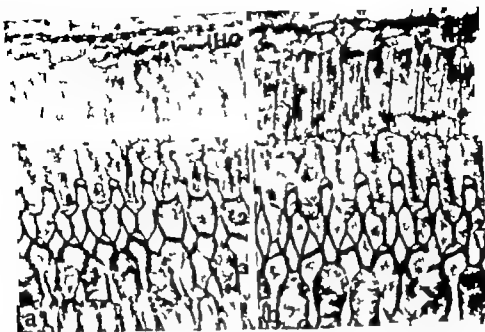


FIG. 6. Surface preparations of organs of Corti from guinea pig treated with kanamycin. (a) Total coil from base. All inner hair cells (IHC) intact (the hairs of the cells are in focus). Total loss of outer hair cells in the first row (1) partial loss in second (2) and third (3) rows. (b) Two and three-quarter coil from base. Somewhat longer exposure time to kanamycin than in (a). All inner hair cells, and all sensory cells in the first and second row degenerated. Only one degenerated sensory cell in the third row (a and b 1978).

maculae. In these animals a considerable loss of sensory cells both in the striola of the macula utriculi and the central regions of the cristae, and a similar loss of cells in the peripheral parts of these sensory epithelia were observed.

The intratympanic application of streptomycin and parenteral injection of kanamycin gave the same pattern of degeneration in the vestibular sensory regions. The relative vulnerability of the sensory cells in the different areas is illustrated in Fig. 5. The sensory cells of type I were more sensitive than those of type II. Among the type I cells, those located in the central regions of the cristae and the striola of the maculae were the most vulnerable.

The degeneration pattern in the cochlea was similar to that seen after streptomycin administration (Fig. 6) however whereas the sensory cells on the cristae ampullares and the macula utriculi generally were more sensitive to streptomycin than the hair cells of the organ of Corti, the latter were generally more sensitive to kanamycin than the vestibular sensory cells. No apparent changes of the statoconium membranes and the cupulae could be seen.



## DISCUSSION

The sensitivity of the sensory epithelia of the inner ear to ototoxic antibiotics is different in different species. Thus it appears from the literature that equivalent doses of e.g., streptomycin produce more pronounced damage in man and cat than in the guinea pig. In fact, in the guinea pig, it is difficult to induce substantial damage to the inner ear sensory epithelia by parenteral application since most of the animals die as a result of general intoxication before such damage can be observed. However, massive degenerative changes in the inner ear can be produced by instilling streptomycin into the middle ear. Spoendlin (1966) using this avenue of administration, in the cat showed that the effect upon the vestibular sensory epithelia was not a result of non specific osmotic changes, but depended upon a direct toxic influence of the streptomycin. In his opinion the streptomycin diffused from the middle ear via the round window membrane into the inner ear.

As early as 12 hours after intratympanic injection Spoendlin (1966) observed, by electronmicroscopy an agglomeration of chromatin in the nuclei of the type I cells. Later loss of ribosomes and endoplasmic reticulum became apparent whereas mitochondria and membranes of nuclei and cells remained normal for some time. The nerve endings and the nerve fibers were also found to be normal. In Spoendlin's opinion the streptomycin attacked primarily the protein synthesis, which is dependent upon the cell nuclei and the ribosomes. On the other hand Wersäll & Hawkins (1962) after parenteral streptomycin injections in the cat observed changes of the mitochondria, swelling and vacuolization of the cytoplasm of the sensory cell prior to pyknosis of the nuclei. Studying the effect of parenteral injections of streptomycin in guinea pig Duvall & Wersäll (1964) postulated that the streptomycin affected primarily the plasma membrane of the cells and the mitochondria.

In the present investigation it was difficult to determine the exact sequences of degeneration as the finer intracytoplasmic changes could not be visualized in light microscopy. Early signs of degeneration were agglomeration of the chromatin in the periphery of the nucleus and sometimes a slight swelling of the nucleus. Later the nuclei became pyknotic or fragmented. Before complete disintegration of the nuclei occurred, changes in the hairs could be noticed. Furthermore an increased number of cytoplasmic protrusions were seen which were similar to those described by Wersäll & Hawkins (1962) and Spoendlin (1966). A final stage was the appearance of the collapse figure. The formation of the collapse figures in the vestibular sensory epithelia corresponded to those described in the organ of Corti (Engström *et al.* 1966).

Some authors have reported regional differences in the sensitivity of the vestibular sensory cells to streptomycin. In the present study a distinct gradation in the sensitivity of the different areas was evident after both

Intratympanic application of streptomycin and parenteral administration of kanamycin. In agreement with findings by McGee & Olaszewski (1962) and Spoendlin (1966) the sensory cells of the cristae were found to be more vulnerable than those of the maculae. Igarashi (1965) and Igarashi *et al* (1966) stated that the administration of streptomycin sulphate is a useful method of selectively suppressing the semicircular canal function and they described a nearly selective damage to the sensory cells of the cristae in squirrel monkey. Such findings, however, were not supported by the present study in guinea pig, as extensive damage to the cristae epithelium was always accompanied by a considerable loss of hair cells in the macula utriculi. Duvall & Wersäll (1964) did not notice any difference in vulnerability between the sensory cells of the crista and those of the macula utriculi in the guinea pig.

In the present study the macula utriculi always showed more extensive changes than the macula sacculi. This is in agreement with observations by Berg (1951) Rüdel *et al* (1951) Hawkins *et al* (1952) and Wersäll & Hawkins (1962).

Many authors have described a more pronounced loss of hair cells on the top of the crista than on its slopes (Berg, 1951; Rüdel *et al*, 1951; Hawkins *et al*, 1952; Wersäll & Hawkins, 1962; McGee & Olaszewski, 1962) a finding which is not in agreement with the present observations. As discussed by Lindeman (1969) it seems justified to divide the sensory epithelium on the cristae into central and peripheral regions. Thus the peripheral part of the sensory epithelium located on the top (vertex) of the crista and bordering the plane semilunata, morphologically as well as with respect to vulnerability to streptomycin and kanamycin corresponds to the peripheral part of the epithelium on the slopes of the crista. In the present study the sensory cells in the central region were more vulnerable than the more peripherally located cells.

Neumann & Neubert (1938) described regional differences in sensitivity within the macula utriculi. They observed degenerative changes which started in the central part of the macula utriculi; however in their opinion, this region did not correspond to the striola.

Both after parenteral and intratympanic application of streptomycin in the cat the type I cell has been reported to be more sensitive than the type II cell (Wersäll & Hawkins, 1962; Spoendlin, 1966). On the other hand Duvall & Wersäll (1964) did not find clear differences in sensitivity of the type I and type II cells in the guinea pig. In the present investigation, the type I cell seemed in general more vulnerable than the type II cells. Most sensitive were those type I cells in the central parts of the cristae and in the striola of the maculae. Again in the background of the highly specialized structure of these regions (see Lindeman, 1969) this observation is most interesting. It supports the suggestion that the function of these regions differs from that of the peripheral parts. It is not yet possible to explain why the cell in the central parts of the cristae and in the striola of the

maculae are particularly sensitive to ototoxic antibiotics however according to Spoendlin (1966) there seems to be a certain correlation between the intensity of the protein metabolism of the sensory cells and their sensitivity to streptomycin.

In the present study the sensory cell loss in the least damaged epithelia was confined to the central parts of the cristae and the striola of the maculae. In future studies of vestibular sensory epithelia, it must be realized that degenerative changes may be restricted to such small areas. In the case of a macula with damage restricted to the striola, a longitudinal section through the striola, will give an exaggerated picture of the extent of damage while an extra striolar section might fail to show any degeneration at all. It is also important to know that in a medially situated part of the macula utriculi, the number of sensory cells per unit of area is less than elsewhere peripherally (Lindeman, 1969). Thus a section through this region may give an erroneous impression of sensory cell loss.

Some authors have tried to explain the vulnerability of the sensory regions in the inner ear to different noxious agents on the background of phylogeny. In a study of "pathological types of cochleo-saccular degeneration" Schuknecht *et al* (1965) concluded "In demonstrating a greater susceptibility to injury the pars inferior of the labyrinth abides by the biological rule that the phylogenetically newer system in an organism generally exhibits a distinctly greater susceptibility to abuse than do older systems." The results of the present study however fails to confirm that statement. Thus, the macula sacculi which together with the cochlear duct represents the phylogenetically youngest part of the labyrinth, turned out to be most resistant to both kanamycin and streptomycin. It must be emphasized that the degeneration pattern after exposure to noxious agents is dependent upon the specificity of the agent. In the present investigation, the hair cells of the organ of Corti were generally more sensitive to kanamycin than the vestibular sensory cells. The sensory cells of the cristae and the macula utriculi on the other hand were generally more sensitive to streptomycin than were the cochlear hair cells. Furthermore Wintner (1968) after X-ray irradiation of the inner ear observed a different pattern of degeneration in the vestibular sensory regions than that reported here.

Berg (1951) reported disappearance of the statoconium membrane after streptomycin intoxication a phenomenon also noted by Kimura & Perlman (1956) after obstruction of the cochlear inferior. They explained the breaking down of the statoconium membranes as a result of extensive changes of the sensory epithelium. In the present investigation no convincing degenerative changes of the cupulae or the statoconium membranes could be noticed however the possibility that the degenerative changes were too small or the observation period too short must still be held open.

## RESUME

L'effet d'application intratympanale de streptomycine et d'injection parentérale de kanamycine sur l'épithélium sensoriel vestibulaire a été étudié en se servant de la technique de préparation de la surface spéculaire. Variation régionale de la sensibilité sont alors trouvées. Les changements dégénératifs se manifestent premièrement dans les parties centrales de l'épithélium des cristaux et de la striola des maculae.

## ZUSAMMENFASSUNG

Die Wirkung von intratympanaler Streptomycin Applikation und parenteralen Kanamycininjektionen auf den vestibulären Sinnesepithelien ist mit der Häutchenmethode studiert worden. Dabei sind klare regionale Unterschiede in der Vulnerabilität gegenüber den oben genannten toxischen Antibiotika gefunden worden. Die Sinnesepithelien der Crista ampullares waren mehr vulnerabel als die Macula utriculi und diese wiederum deutlich mehr vulnerabel als die Macula sacculi. Die degenerativen Veränderungen zeigten sich hauptsächlich in den zentralen Teile des Crista epithelium und in der Striola der Maculae.

## REFERENCES

- DRACH, C., and KRAMER, P. 1962 Experimentelle und klinische Untersuchung über die Otolotoxizität von Kanamycin. *Arch Ohren Nasen Kehlkopfheilk* 170: 534.
- ELIAS, R. 1955 The toxic effect of streptomycin on the eighth cranial nerve. *Ann Otol Rhinol Laryng* 64: 468.
- 1951 The toxic effect of streptomycin on the vestibular and cochlear apparatus. *Acta Otolaryng (Stockh.) Suppl* 87.
- GAUL, H. 1949 Lésion toxique vestibulaire et cochléaire de la streptomycine. *Revue de neurologie* 100: 318.
- HALL, J. J. and KRAMER, J. 1964 Streptomycin poisoning ear sensory cells. *Acta Otolaryng (Stockh.)* 3: 381.
- ELIAS, R., AMER, H. W. and ANDERSON, L. 1966 *Structure and function of the organ of Corti*. Almqvist & Wiksell, Stockholm.
- HALL, J. E. JR. and ANDERSON, H. 1966 Effect of kanamycin on cochlear cytoarchitecture. *Acta Otolaryng (Stockh.)* 3: 391.
- HALL, J. J. and KRAMER, J. 1952 The ototoxicity of streptomycin. *Acta Otol Rhinol Laryng* 61: 789.
- KRAMER, M. 1965 Temporary suppression of semicircular canal function in squirrel monkeys after streptomycin sulfate. The role of the vestibular organ in the perception of space. p. 273-283. SP-77. National Aeronautics and Space Administration, Washington, D.C.
- KRAMER, M., M. LAM, M. F. and GRAYBIEL, A. 1966 Clinical pathological correlations in squirrel monkey after suppression of semicircular canal function by streptomycin sulfate. *Acta Otolaryng (Stockh.) Suppl* 314.
- KRAMER, L. 1963 Effect of some ototoxic drugs upon the pattern and innervation of cochlear sensory cells in the guinea pig. *Acta Otolaryng (Stockh.) Suppl* 289.
- KRAMER, L. 1967 Cellular pattern and innervation of the vestibular sensory epithella. *Acta Otolaryng (Stockh.)* 3: 391.
- 1965 Studies on the morphology of the sensory region of the vestibular apparatus. *Folia Anat. Entomol.* In press.

- McGEE, M. T. and OLSZEWSKI, J. 1962: Streptomycin sulfate and dihydrostreptomycin toxicity: Behavioral and histopathologic studies. *Arch. Otolaryng.* (Chic.) 75, 235.
- NEUMANN, H. and NEUBERT, K. 1958: Die Sensularien des Innenohres unter der Einwirkung von Streptomycin. *Arzneimittelforschung* 8, 63.
- RÜDEL, L., FUNNEN, W., GRAY, H., LÜTHY, F., NAGER, G. and TACHIBANA, B. 1951: Weitere Befunde über die toxischen Wirkungen von Streptomycin und Chinin am Gehörorgan des Meerschweinchen. *Bull. Schweiz. Akad. Med. Wiss.* 7, 276.
- SCHUMMECHT, H. F., IGARASHI, H. and GACEK, R. R. 1965: The pathological types of cochleo-saccula degeneration. *Acta Otolaryng.* (Stockh.) 59, 154.
- SPOENDLIN, H. 1966: Zur Ototoxizität des Streptomycins. *Pract. Otorhinolaryng.* (Basel), 28, 305.
- WERNICK, J. and HAWKINS, J. E. JR. 1962: Vestibular sensory epithelia in cat labyrinth and their reactions in chronic streptomycin intoxication. *Acta Otolaryng.* (Stockh.) 51, 1.
- WINTNER, F. O. 1968: Personal communication.

*Author's present address*

*Et Lilin Lasarettet SÄffle Sweden*

## DISCUSSION

*J. Friedmann* I have admired the brilliant technique. What is the mechanism of the collapse? Are perhaps the desmosomes affected? The detailed study of the underlying process requires the aid of electron microscopy.

*G. Kelemen* Two points. (1) The role of spontaneous (re-experimental) pathology in animals. (2) Mixed data on ototoxic antibiotics.

*M. Portmann* Très intéressé par cette communication ma question est la suivante: Pensez-vous qu'il y a une relation entre les zones lésées et les zones de fonction maximum des maculae? Par exemple dans l'organe de Corti ce sont les cellules externes du premier tour qui sont les plus affectées c'est à dire celles qui sont les plus stimulées par les vibrations. La toxique en bloquant le système enzymatique cellulaire fragilise donc la cellule vis à vis du stimulus. On peut donc supposer que la localisation n'est pas spécifique du toxique mais spécifique du fonctionnement.

*H. Engström* Mr Kelemen has touched upon a most important problem, that is, the question of normal and pathological. In guinea pigs the inner ear is extremely regular in structure, but this is not always true for monkeys, and for human beings over 30-40 there is always a rather pronounced irregularity.

*H. H. Lindeman* (Reply) to Mr Friedmann: The collapse figures have been described in the organ of Corti by Engström *et al.* (1966). The mechanism in the formation of the collapse figures in the vestibular sensory epithelia seems to be similar. After disintegration of the cytoplasm and the cell nucleus, the plasma membrane "collapses" and loses its attachments to surrounding supporting cells, except in certain areas, where the attachments are especially tight. This gives the characteristic spider-like appearance of the collapse figure as seen on surface preparations.

Of course I agree with you that the surface preparation technique is not the only way to study inner ear sensory epithelia. A combination of different methods is always superior to the use of one single method. However, for certain types of investigations, the surface preparations are especially well adapted. The method is fast and reliable. It allows identification of virtually every sensory

and supporting cell within a sensory region, and is, therefore, useful for quantitative studies. The method also permits exact orientation, which is a prerequisite for systematic studies of corresponding regions in different animals.

**T. Mr. Kelemen:** Before studying the effect of ototoxic agents upon the vestibular sensory epithelia, the structure of these epithelia was studied in more than 100 normal guinea pigs, a background which was of great help in identifying abnormalities which appeared after treatment with ototoxic antibiotics.

**T. Mr. Portmann:** The clear regional differences in the normal structure of the vestibular sensory epithelia, in addition to the regional differences in sensitivity to ototoxic antibiotics, strongly suggest a different function of the central and peripheral areas of the cristae and maculae. However, as far as I know, no functional studies have been made with this suggestion in mind, and it is therefore not possible to draw any conclusions regarding this matter at the moment.

- McGEE, M. T. and OLAKIWSKI, J., 1962 Streptomycin sulfate and dihydrostreptomycin toxicity: Behavioral and histopathologic studies. *Arch. Otolaryng.* (Chic.) 75, 291.
- NEUMANN, G. and NEUBERT, H., 1938 Die Sensularien des Innenohres unter der Einwirkung von Streptomycin. *Arch. f. d. ges. u. exp. Med.* 8, 63.
- ROEDIGER, L., FÜRBERG, W., GRAP, H., LÜTHY, F., NAGEL, G. and TACHIKAWA, B., 1951 Weitere Befunde über die toxischen Wirkungen von Streptomycin und Chinin am Gehörapparat des Meerschweinchens. *Bull. Schweiz. Akad. Med. Wiss.* 7, 276.
- SCHLICKMANN, H. F., JOHANSSON, M. and GACKER, R. H., 1965 The pathological types of cochleo-saccular degeneration. *Acta Otolaryng.* (Stockh.) 59, 154.
- SPOENDLIN, H., 1966: Zur Ototoxizität des Streptomycins. *Pract. Otorhinolaryng.* (Basel), 23, 305.
- WENSALL, J. and HAWKES, J. E. JR., 1962: Vestibular sensory epithelia in cat labyrinth and their reactions in chronic streptomycin intoxication. *Acta Otolaryng.* (Stockh.) 51, 1.
- WINTNER, F. O., 1968 Personal communication.

*Author's present address*

Kir. klin. Lasarettet Söfste Sverig.

## DISCUSSION

**J. Friedmann** I have admired the brilliant technique. What is the mechanism of the collapse? Are perhaps the desmosomes affected? The detailed study of the underlying process requires the aid of electron microscopy.

**C. Kelemen** Two points. (1) The role of spontaneous (re-experimental) pathology in animals. (2) Mixed data on ototoxic antibiotics.

**M. Portmann** Très intéressé par cette communication ma question est la suivante. J'enseigne vous qu'il y a une relation entre les zones lésées et les zones de fonction maximum des macula? Par exemple dans l'organe de Corti ce sont les cellules externes du premier tour qui sont les plus affectées c'est à dire celles qui sont les plus stimulées par les vibrations. La toxique en bloquant le système enzymatique cellulaire fragilise donc la cellule vis à vis du stimulus. On peut donc supposer que la localisation n'est pas spécifique du toxique mais spécifique du fonctionnement.

**H. Engström** Mr Kelemen has touched upon a most important problem, that is the question of normal and pathological. In guinea pigs the inner ear is extremely regular in structure but this is not always true for monkeys, and for human beings over 30-40 there is always a rather pronounced irregularity.

**H. H. Lindeman** (Reply) to Mr Friedmann The collapse figures have been described in the organ of Corti by Engström *et al.* (1966). The mechanism in the formation of the collapse figures in the vestibular sensory epithelia seems to be similar. After disintegration of the cytoplasm and the cell nucleus, the plasma membrane "collapses" and loses its attachments to surrounding supporting cells, except in certain areas, where the attachments are especially tight. This gives the characteristic spider-like appearance of the collapse figure as seen on surface preparations.

Of course I agree with you that the surface preparation technique is not the only way to study inner ear sensory epithelia. A combination of different methods is always superior to the use of one single method. However for certain types of investigations, the surface preparations are especially well adapted. The method is fast and reliable. It allows identification of virtually every sensory

Simplex virus, reovirus, and unidentified infectious particles, has suggested a viral aetiology in Burkitt's lymphoma. In addition Burkitt's lymphoma cells have been successfully identified in cultures used to detect antibodies in human serum. In view of these discoveries, it is conceivable that nasopharyngeal carcinomas might be similarly caused by a virus.

The purpose of this communication is to report on the results of preliminary studies on viral isolation and tissue culture from material obtained from nasopharyngeal carcinomas in Chinese patients. Serological investigations and epidemiology are also considered.

### Tissue cultures

Little information is available in the literature on tissue culture of nasopharyngeal carcinomatous material.

Burke (1967) in Singapore, submitted five specimens obtained from primary growths of nasopharyngeal carcinoma to tissue culture. He was able to maintain all growths and multiplication of explants *in vivo* for a maximum of 35 days, with an average survival time of 30 days. The four metastatic cervical lymph node tissues all failed to grow. All the five cases showed spontaneous degeneration followed by death. It is emphasized that the demonstration of viruses in human tumour tissues does not necessarily mean a causal relationship.

### Present Series

Tissues obtained from the primary growth of 10 histologically proven and untreated epidermoid carcinomas of the nasopharynx were sent to Dr J Huebner for tissue culture and viral isolates. Only one specimen survived in culture for a short period. It is most likely that distance and difficulties in transportation and delivery contributed to this failure as the nasopharyngeal carcinoma cells did not arrive in good condition. Improved packing and more reliable shipping procedures are under consideration. This line of research will be continued.

Table 1 gives the adenovirus incidence demonstrated serologically amongst Chinese nasopharyngeal carcinoma patients compared with the

TABLE 1 *Comparative findings of adenovirus studies*

	Hong Kong virus isolates (1966-1967)	Serological positives (N.P.C.)	Total no. of cases	Percentage
Type adenovirus				
2	4	6	47	12.77
3	10	0	47	—
8	1	0	47	—
7	3	19	47	40.43
12	0	2	47	17.02



## VIRUS AS THE CAUSE OF RHINOPHARYNGEAL CARCINOMA

D LAING

*Hong Kong*

Tissue cultures from the primary growths of 10 nasopharyngeal carcinoma patients failed to grow. Nasopharyngeal carcinoma patients in Chinese yielded more evidence of infection in adenovirus types 7 and 12 than the normal Chinese population. Sera from 27 Chinese nasopharyngeal carcinoma patients were tested for precipitating antibody to antigen prepared from Burkitt's lymphoma cells. There was a high incidence 85% of positive reactions in these patients. The possibility that nasopharyngeal carcinoma has a viral cause or relationship with Burkitt's lymphoma is discussed. While there is no evidence to support this contention the possibility can not be excluded of a predisposing virus infection as the initiator of a malignant process or transformation.

I have been asked to express my views on the viral aetiology of rhinopharyngeal carcinoma. Let me say at once I am not a virologist and my knowledge of this subject is extremely limited, but my interest in the study of the possible connection of viruses in this neoplasm has been stimulated not only by the dearth of information relating to it but also by the failure to find a known cause for this unique and recalcitrant growth.

In the past there was wide acceptance of the hypothesis that inhaled exogenous, environmental carcinogen was an important aetiological factor. However there was insufficient scientific evidence to support this contention since no known offending carcinogen has been found. The ethnic factor in this disease has received increasing recognition because of the characteristic epidemiological features and high incidence in the Mongoloid races of South East Asian countries and related communities. In addition, this disease predominates in all communities where Chinese congregate outside their homeland.

Considerable and rapid advances in virology have been made since the pioneering discoveries of Ellerman & Bang (1908) five decades ago. The relationship between viruses and tumours has been extensively studied by many virologists whose significant and classical contributions, in combination with the discovery of tissue culture and the development of electron microscopy have made it possible to isolate and study the structure and properties of viruses and investigate intracellular viral behaviour and events.

At present, no human cancer has yet been proved to derive from any virus, but two human adenoviruses, types 12 and 18 have been found to produce tumours in hamsters and mice. Recently the isolation of Herpes

TABLE 3. Comparison of results with Old *et al*

Author	Positive sera			
	Burkitt lymphoma	N.P.C.	Other carcinoma and healthy controls	
Old <i>et al</i> , 1966	56	Africans 74 U.S.A. 95	11 12	
This series, 1968		Chinese 83		

series is concerned only with southern Chinese and represents the first observation of its kind relative to this serological overlap.

The incidence is 83% compared to Old's African figure of 74% and north American of 95% positives for this precipitating antibody as compared to a control incidence of between 11 and 12%. It is suggestive of a serological kinship between Burkitt's lymphoma and nasopharyngeal carcinoma, but, as to what this exact relationship is, only further serology study can help.

It is possible that this high incidence of positive sera in patients with Burkitt's tumour and in nasopharyngeal carcinoma in the Chinese may not be confined to these two neoplasms.

All the 27 cases were histologically proven to be epidermoid carcinomas of the primary growth. When the blood samples were taken, the condition of all these cases was quiescent: there was no local recurrence, palpable cervical lymph node or distant metastases. Their survival period ranged from 10 months to 8 years and 8 months.

The four negative series survival period varied from 2 to 7 years. Only one of these received combined radiotherapy and chemotherapy. Of the 23 positive sera, 10 were treated with radiotherapy only and 13 received combined radiotherapy and chemotherapy. The survival period varied from 10 months to 8 years and 8 months. All the five adult control cases were negative.

From a study of this small number of cases, there was no correlation between stage of the disease, survival period, type of therapy and positive and negative sera.

### *Epidemiology*

It has been demonstrated and accepted that the southern Chinese have a striking preponderance of rhinopharyngeal carcinoma. Ho's analysis of a large series of cases found that the frequency rates differed in different regions of South China: the rates in Kwangtung are higher than those in

TABLE 2 *Preliminary complement fixation test*

Tumour antigen studies in 16 Chinese nasopharyngeal carcinoma patients

Tumours	Positive	Negative	No. of tests done
Adenovirus 7 #4	5	11	16
SV 40 #	1	10	11
Polyoma #3	1	0	7
Adenovirus 7 (FT 1160)	3	15	1
Polyoma tumour #4	7	16	18

adenovirus isolates in normal individuals in Hong Kong, carried out by the Government Microbiological Department during two years. The inference is that the nasopharyngeal carcinoma patients yield more evidence of infection than many of the Hong Kong population. All results which were "trace positive" were disregarded. If these were added, the evidence is further strengthened in adenovirus types 7 and 12, in each of which six "trace positives" were found.

Bearing in mind that no paired sera investigations were done, and comparing the few positive Hong Kong population isolates during the two years, and the definitely higher serological positives for adenovirus types 7 and 12 in nasopharyngeal carcinoma patients, more extensive studies into this aspect are indicated. It is emphasized that the numbers are too small to draw any worth while conclusions.

In an earlier group 16 nasopharyngeal carcinoma patients sera were studied, using assorted tumour antigens by the complement fixation test. The results are equivocal and a particular feature was that where SV 40 or adenovirus positives occurred among this early group positives also occurred in equal dilutions with normal rat cell clones and hamster cells also. Other tumour antigens than the ones in the table yielded constantly negative findings for antibodies, among them being adenovirus 12 10 adenovirus 31 0 and respiratory syncytium virus. So far all efforts to demonstrate any antibodies to tumour antigens have failed in the complement fixation test. The significance is that this, at the present time indicates an unrewarding yield.

In view of recent findings that indicated a serological overlap between Burkitt's lymphoma and other tumours, a study of 27 Chinese nasopharyngeal carcinoma cases was made. A search was undertaken for a precipitating antibody in nasopharyngeal carcinoma patients sera tested against Burkitt's lymphoma tumour antigen. A positive precipitating antibody was found in 23 out of 27 of these patients, with four negatives, giving 85% positive.

A comparison with Old *et al* (1968) is more interesting than this figure would suggest because Old drew the sera from Africans and patients in United States of America, but does not record results from Asiatics. This

TABLE 5 Chinese land and marine populations in Hong Kong in 1961 by age and sex

Population	Under 25 yrs			25 yrs and over			All ages, M + F
	M	F	M + F	M	F	M + F	
Land	807,572	729,917	1,537,489	688,590	707,211	1,405,801	2,943,290
Marine	43,420	30,297	82,717	29,101	21,763	50,864	136,581
Total	850,992	760,214	1,610,206	717,691	728,974	1,446,665	3,079,901

in a population already known to have a high incidence of this disease and has led me to search for possible causal factors—environmental, genetic or viral. Table 5 shows the Chinese land and marine population in Hong Kong in 1961 by age and sex. Table 6 shows a comparison between the incidence of nasopharyngeal carcinoma among the "boat people" and the rest of Hong Kong.

Hong Kong has a marine population of 136,581. It is descended from mainland Kwangtung. The people live mainly in their crafts of various sizes, earning their livelihood by fishing. Some are seamen and a minority have shore employment but continue to live in houseboats, where often two or three generations of a family share a common environment. The majority of this population is Tan ha and have been residents of Hong Kong for hundreds of years. They are highly religious and superstitious. They congregate in small communities in the various bays and fishing villages both in the Island and on the Kowloon Peninsula. Intermarrying amongst these settlers is shown by the limited number of family names. The illiteracy rate is high. Shore dwellers do not look favourably on marriage with the Tan ha's. During the last 10 years their standard of living has improved considerably. The majority is physically healthy, robust, and hardy. The houseboat is kept scrupulously clean. Ventilation and natural light in the cabins are poor. The floor area in each cabin varies from 20 to 40 sq. ft. The "boat people" spend most of their time on deck in the open-air where the atmosphere is anything but dusty. Kitchen smoke is not an irritating factor, a good outlet for fumes being provided in the kitchen. Butane gas is now used in the larger crafts. Incense smoke from the burning of joss sticks in the small altar is considerably less than in other homes because of economic reasons. Worshipping is generally done by the female. Neither inhalation of snuff nor opium is indulged in by these people. Tobacco chewing is rare but the use of water pipes is common. Diet consists mainly of sea food, rice, small quantities of red and white meat, and large quantities of vegetables, and is roughly estimated at 2000 to 2500 calories daily. A comparative retrospective study of the ways of life of 15 nasopharyngeal carcinoma cases amongst the Tan ha's with the same number from the land dwellers of more or less similar socio-economic

TABLE 4 *Results of A P C patients sera for precipitating antibody to antigen prepared from cultured Burkitt's lymphoma cells*

No	Name	Age	Sex	Type of growth	Stage of disease	Radio-therapy	Chemo-therapy	Survival period	Result	Serum	
										Positive	%
1	L. S. V	43	M	Epidermoid ca.	3	yes	no	3 yrs 8 months	Decreased		
2	F. W. H.	49	F	Epidermoid ca.	2	yes	yes	2 yrs	Alive	+	
3	L. Y. L.	46	M	Epidermoid ca.	1	yes	no	7 yrs	Alive	+	
4	H. C. W.	41	M	Epidermoid ca.	3	yes	no	3 yrs	Alive	+	
5	L. S.	33	M	Epidermoid ca.	3	yes	yes	1 yr 3 months	Alive	+	
6	L. M. H.	41	M	Epidermoid ca.	3	yes	yes	1 yr	Alive	+	
7	C. L. W.	39	M	Epidermoid ca.	3	yes	yes	2 yrs	Alive	+	
8	V. H.	40	M	Epidermoid ca.	3	yes	yes	1 yr 8 months	Alive	+	
9	C. L. Y.	30	F	Epidermoid ca.	3	yes	no	2 yr 8 months	Alive	+	
10	L. F. T.	44	M	Epidermoid ca.	3	yes	yes	3 yrs	Alive	+	
11	W. C.	47	M	Epidermoid ca.	3	yes	yes	2 yrs 3 months	Alive	+	
12	M. S. P.	43	M	Epidermoid ca.	3	yes	yes	5 yrs	Alive	+	
13	S. K. H.	46	M	Epidermoid ca.	2	yes	no	7 yrs	Alive	+	
14	Y. B. T.	32	M	Epidermoid ca.	2	yes	no	3 yrs	Alive	+	
15	W. Y.	38	F	Epidermoid ca.	3	yes	yes	2 yrs	Alive	+	
16	C. H.	42	M	Epidermoid ca.	3	yes	yes	1 yr 3 months	Alive	+	
17	C. K. W.	31	M	Epidermoid ca.	1	yes	no	4 yrs	Alive		
18	L. S. K.	20	F	Epidermoid ca.	2	yes	no	3 yrs 10 months	Alive	+	
19	S. C. T.	60	M	Epidermoid ca.	3	yes	yes	2 yrs	Alive		
20	N. P. K.	39	M	Epidermoid ca.	2	yes	no	3 yrs 9 months	Alive	+	
21	L. K. S.	41	M	Epidermoid ca.	3	yes	no	2 yrs 11 months	Alive		
22	L. H.	42	M	Epidermoid ca.	2	yes	no	8 yrs 8 months	Alive	+	
23	C. K. W.	55	M	Epidermoid ca.	4	yes	yes	1 yr 8 months	Alive	+	
24	C. S.	43	M	Epidermoid ca.	3	yes	no	8 yrs 3 months	Alive	+	
25	Y. C. Y.	30	M	Epidermoid ca.	3	yes	no	3 yrs	Alive	+	
26	K. Y. K.	28	F	Epidermoid ca.	1	yes	yes	2 yrs	Alive	+	
27	L. K. S.	64	F	Epidermoid ca.	3	yes	yes	10 months	Alive	+	

the central provinces of Chekiang Fukien and Kiangsu. Further he showed amongst the Hong Kong-domiciled group a significantly higher incidence in the floating population" which has a crude incidence rate of 124 per million as against 78 per million in the land population.

This striking difference shows the existence of a localised risk community

## Adenoviruses and Tumours

These are of particular interest, especially types 12 and 18, which were originally isolated from degenerated human adenoid tissue exhibiting spontaneous degeneration in culture. One of the properties of these viruses is, in some cases, that they cannot be recovered from the tumour they produced, and it has been suggested that the virus, after initiating a neoplastic conversion of the normal cell, disappears—a process analogous to the behaviour of the bacteriophages. Could this be the reason why the search for human tumour virus has been unsuccessful so far?

It is interesting to note that in spite of the frequency of upper respiratory tract infection in Hong Kong, especially in the low socio-economic group, adenovirus types 12 and 18 have not been isolated in 2 years, but eight adenovirus type 12 serological positives were found out of 47 nasopharyngeal carcinoma patients.

Another significant clinical finding is that in 6307 cases of nasopharyngeal carcinoma patients case records, obtained from colleagues in Singapore, Taiwan, and Hong Kong, not a single one was adenoidectomized. As adenoidectomy is comparatively rarely performed on Chinese children, no connection or estimation can be made of its relation to the development of nasopharyngeal carcinoma. However, it may be a clue worth following up.

In the light of this very restricted research, it is not possible to express any conclusion on this complex problem, and further investigations will be done.

## CONCLUSION

1. Tissue culture from the primary growth of 10 nasopharyngeal carcinoma patients failed to grow.

2. Chinese nasopharyngeal carcinoma patients yield more evidence of infection in adenovirus types 7 and 12 than the normal Chinese population.

3. Sera from 27 Chinese nasopharyngeal carcinoma patients was tested for a precipitating antibody to antigen prepared from Burkitt's lymphoma cells. There is a high incidence, 85% of positive reactions in these patients.

4. A study of the floating population in Hong Kong showed no evidence that nasopharyngeal carcinoma is vector-borne in this high-risk population.

## ACKNOWLEDGMENT

I would like to express my grateful thanks to Dr Robert J. Huebner, Chief, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md., and Dr Herbert F. Oetgen of the Sloan-Kettering Institute for Cancer Research, not only for their help in making this work possible, but also for doing all the serological investigations free of charge. I am indebted to Dr S. D. Stanton, my co-worker in this research, for his encouragement and contributions of material used in this paper. I wish to thank Dr John Grant for advice in the preparation of the manuscript.

TABLE 6

 $p = 0.001$ 

Year	No of case		
	All Chinese	Boat people	Others
1936	95	4	91
1937	160	12	148
1938	252	4	253
1939	261	12	219
1960	291	10	284
1961	360	19	342
Total	1 433	61	1,372
1961 population	3 079,901	81 649	2,998,252
Cases/million/annum	8	124	6

nonic standard was conducted. There is no evidence to suggest that the high incidence rate is due to any difference in the environment nor is there increased hazard in their environment. It is a matter of conjecture that intermarriage might be a genetic factor since it is postulated that inherited traits may determine a degree of susceptibility.

Malaria is a notifiable disease, now successfully controlled. Sporadic cases occur in both rural and urban areas. The immediate environment of the boat population does not favour the breeding of mosquitoes. Yellow fever is unknown. There is no evidence to suggest or support the hypothesis of a possible vector borne viral aetiology for nasopharyngeal carcinoma in this community.

Four histologically confirmed cases of Burkitt's lymphoma have been recorded in Hong Kong: none came from the floating population but they were born in Hong Kong and have never left the colony.

The unconfirmed hypothesis that Burkitt's lymphoma has a viral cause has led to the possibility that nasopharyngeal carcinoma might be similarly implicated by a virus. Since Burkitt's lymphoma is a variant of lymphosarcoma and reticulo-cell sarcoma, and this diagnosis is often confused with nasopharyngeal carcinoma, one might ask if nasopharyngeal carcinoma could be related to Burkitt's lymphoma. In Hong Kong almost all cases are carcinoma and the analogy does not suggest itself.

To say that there is no evidence that nasopharyngeal carcinoma has any relationship to Burkitt's lymphoma does not of course exclude the possibility of a viral cause for nasopharyngeal carcinoma, which remains a possibility. It is again possible that a predisposing virus infection might well be one causing slight or no symptoms. In Hong Kong reovirus has not been isolated up to the present.

*L. B. W. Jongkees* Mr Laing I probably the best connoisseur and has seen more patients suffering from rhinopharyngeal carcinoma than anybody else. In Holland and in Indonesia cases are seen, but the greatest percentage is in Chinese (pure and mixed) people. Might it be possible that a transmission of virus from the mother takes place? We know from the studies of Biters that this takes place in mice with breast cancer (the so called milk factor).

*J. McAuliffe Curtin* In Mr Laing's large clinical material of this particular problem, could he say what is the ratio of males to females? Also, what is the prognosis in relation to the onset of symptoms with regard to the parent's age?

*D. Laing* (Reply) to Mr McAuliffe Your question whether there is a familial tendency to this disease is a most interesting one. In my series of over 1600 cases, there were many incidences of two members of a family suffering from this disease, and even in some families there were three members affected. The finding in one family is highly suggestive of a familial tendency. There were seven offspring, four females and three males. The mother died of natural causes, the father of nasopharyngeal carcinoma, two males and two females died of the same disease, two other females both having histologically confirmed N.P.C. are still alive and one male is free from this disease.

Two or three members of a family having malignant neoplasms of different organs are not uncommon, and these are most likely coincidental, but seven members of one family having neoplasms in identical anatomical sites, which makes the coincidental explanation most unlikely. It is possible that inherited traits may determine a degree of susceptibility to this disease.

*T. Mr Jongkees* I will deal with your first question of racial susceptibility that Indonesians having Chinese blood in their ancestry also have a high incidence of this disease. This is true not only in Indonesia, but also in Thailand, Vietnam, Burma, and the Philippines, but in Japan this disease is relatively uncommon, and the incidence rate is comparable to that of the western countries. Similarly the Indians and Pakistanis of Caucasian stock have a low incidence rate.

Your suggestion of a carcinogenic milk factor like that of Biters, in mice in nasopharyngeal carcinoma, is a most interesting one. I must admit I have not thought of this approach, and I am sure it is worth following up.

*T. Mr McAuliffe Curtin* The third question concerned the male and female ratio in this disease. In my series it was 2.5:1.

Whilst discussing etiology I wish to add that the 30-39 age group constituted about 80% of the 40-49 age group being most common making up about 34%. Is there any relationship between age of the disease and prognosis? Early diagnosis of this disease before the appearance of cervical lymph nodes or neuro-ophthalmic symptoms, is the key to a good prognosis.

In stage 1 and stage 2 cases, the five-year survival rate is only 40%. Early diagnosis is based on recognition of the nasopharyngeal and otologic syndrome which I described about 15 years ago. The first sign of this is the repeated appearance of blood-streaked aspirated postnasal secretions, combined with unilateral conductive deafness. Nasal obstruction is not an early symptom.

Finally a southern Chinese has suggested either the nasopharyngeal or otologic manifestation of this syndrome is assumed to have nasopharyngeal carcinoma until proved otherwise.



## ZUSAMMENFASSUNG

Versuche Gewebekulturen von Primörgeschwülsten von 10 Patienten mit nasopharyngealem Karzinom zum Weiterwachsen zu bringen, missglückten. Untersuchungen an chinesischen Patienten mit nasopharyngealem Karzinom zeigten ein relativ höheres Auftreten von Infektionen durch Adenovirus Typ 7 und 12 als die normale chinesische Population. Des weiteren wurden die Seren von 27 chinesischen Patienten mit nasopharyngealem Karzinom auf die Bildung von Antikörpern gegen Antigenpräparate aus Burkitt's Lymphoma-Zellen getestet. Das Testresultat zeigte bei diesen Patienten eine sehr hohe Quote von positiven Reaktionen (85%). Im Zusammenhang damit wird die Möglichkeit der Verursachung von nasopharyngealen Karzinomen durch Virus oder in Verbindung mit Burkitt's Lymphoma diskutiert. Eine derartige Annahme lässt sich aus den Befunden nicht stützen, doch kann die Möglichkeit einer prädisponierenden Wirkung einer Virusinfektion als Initiator maligner Prozesse oder Transformationen nicht ausgeschlossen werden.

## REFERENCES

- BELL, T M, MASSIE, A., ROSS, M G R, SIMPSON, D I D and GRIFFITH, E., 1964: *Brit Med J* 1 1514.  
 BELL, T M, MASSIE, A, ROSS, M G R and WILLIAMS, M C., 1964: *Brit Med J* 1 1212.  
 BURKE, T. 1967: UICC Mono Series, *Cancer of the nasopharynx* 1 268.  
 BURKITT D. 1958: *Brit J Surg* 46 218.  
 BURKITT D. and DAVIES, J V B. 1961: 235 367.  
 ELLERMANN V. and BAAG, O. 1908: *Centralb F Bakt L. Abt Orig* 46 593.  
 EPSTEIN M. A. and BARR, V M. 1964: *Lancet* 1 262.  
 EPSTEIN, M. A. ACHO, G. B. G. and POMEY, J H. 1967: *Brit Med. J* 2 290.  
 HELLEMAN M R., and WERNER, J H. 1954: *Proc Soc Exp Med* 85 183.  
 HO, H C., 1968: UICC Mono Series 1 38 63.  
 — 1968: Personal communication.  
 HUEBNER, R J. 1968: Personal communication.  
 HUEBNER, R J, ROWE, W P, TURNER, H C., and LAKE, W T. 1963: *Proc Nat Acad. Sci* 50 379.  
 HUEBNER, R J, ROWE, W P, WARD, T G, PARROTT R H. and BELL, J A. 1954: *New Eng J Med* 251 1077.  
 OTTOCK, H F. 1968: Personal communication.  
 OLD, L. J, BOTES, E. A, OTTOCK, H F, HARTYK E. DE, GREENING, H, WILLIAMS B., and CLIFFORD, P. 1966: *Proc Nat Acad Sci* 56 1699.  
 ROSE, P. 1911: *J Exper Med* 13 397.  
 ROWE, W P, HUEBNER, R J, GILMORE, L. H., and PARROTT R H. 1953: *Proc Soc Exp Biol Med* 84 870.  
 SUTTON, J D. 1968: Personal communication.  
 YABE, Y, S. MURAKAMI, L., BRADY, E., TAYLOR, G. and TRENTIN, J J. 1964: *Science* 143 46.

50 Gloucester Building,  
 Hong Kong

## DISCUSSION

A. Michlke: Is there any family predisposition to this disease? Does it exist or not in the Chinese population?

TABLE 1. ATPase activities in various cochlear structures

Means  $\pm$  h.s.e.

Structure	Na-K-ATPase		Mg-ATPase (moles/kg dr wt/hr)	Mean activity $\pm$ turn no.
	Moles/kg dr wt/hr	% of total ATPase		
Tegm. vasculosum	$5.10 \pm 0.06$	$39 \pm 3$	$2.10 \pm 1.10$	—
Stria vascularis	$.83 \pm 0.43$	$59 \pm 1$	$3.57 \pm 0.41$	1, 2, 3
Lig. spirale				
(a) Behind stria vas.	$0.40 \pm 0.09$	$53 \pm 7$	$0.36 \pm 0.09$	2, 3
(b) Comprising prom. spir. and sulcus externus	$1.60 \pm 0.39$	$56 \pm 6$	$1.26 \pm 0.43$	2, 3
(c) Bordering scala tymp.	$0.33 \pm 0.07$	$36 \pm 7$	$0.63 \pm 0.07$	2, 3
Reissner's membrane	$0.33 \pm 0.06$	$28 \pm 4$	$0.91 \pm 0.10$	1, 2, 3, 4
Organ of Corti	$0.47 \pm 0.27$	$11 \pm 6$	$2.79 \pm 0.79$	2, 3

by incubation in media, prepared with buffers in the pH range from 6.2-9.6.

The role of Na-K-ATPase in the generation of the cochlear potentials (cochlear microphonic potential and +endolymphatic potential) was determined by continuously perfusing the scala vestibuli of the guinea pig cochlea with Krebs Ringer solution during 45 minutes (10  $\mu$ l/min) with and without ouabain ( $10^{-5}$ – $10^{-3}$  M). In Table 1 the distribution of the Na-K-activated and Mg-activated ATPase activities in the cochlea is demonstrated. The stria vascularis and its homologous structure in the chicken, the tegmentum vasculosum, revealed a very high Na-K-ATPase activity. The Na-K-ATPase activity of Reissner's membrane, organ of Corti, and ligamentum spirale, except part b of this structure comprising promontoria spiralis and sulcus externus, is very low. Table 2 gives the activity of the stria vascularis in various turns. There is a decrease for both ATPase activities from the basal to the apical turn; this has also been demonstrated for

TABLE 2. ATPase activity in various cochlear turns

Means with s.e.

Turn no.	Na-K-ATPase		Mg-ATPase (moles/kg dr wt/hr)	Total dry weight of stria vasc., mg
	Moles/kg dr wt/hr	of total ATPase		
1	$6.37 \pm 0.40$	$56 \pm 3$	$5.01 \pm 0.46$	12
2	$7.03 \pm 0.87$	$61 \pm 4$	$4.32 \pm 0.35$	12
3	$4.60 \pm 0.72$	$57 \pm 6$	$3.60 \pm 0.59$	7
4	$4.33 \pm 0.85$	$54 \pm 1$	$2.14 \pm 0.30$	3

## CATION TRANSPORT AND COCHLEAR FUNCTION

W. KUIJPERS

*From the Department of Otolaryngology University of Nijmegen, Nijmegen, Netherlands*

Na-K activated ATPase was determined in the cochlear structures by ultramicro-analytical methods. The highest activity was found in the stria vascularis, with a decrease from base to apex. Ouabain, which inhibits the isolated enzyme system appeared to diminish the CMP and +EP to the same extent. These findings strongly suggest that the Na-K ATPase enzyme system in the stria vascularis plays the key role in the maintenance of the cochlear cationic gradients, while such a role appears highly unlikely for Reissner's membrane. The CMP and +EP appear to depend on the functioning of this pump system.

As shown by many investigators, there is a characteristic difference between the ionic composition of the cochlear fluids (Fernández, 1967). The ionic composition of the perilymph is similar to that of other extracellular fluids. The endolymph—being an extracellular fluid—has an ionic composition similar to that of intracellular fluids.

It is now generally accepted that the maintenance of those cationic gradients in most animal cells depends on the activity of a Na-K-activated ATPase that is specifically inhibited by cardiac glycosides (Skou, 1964).

In view of the cationic gradients in the cochlea between endo- and perilymph we have tried to determine whether these gradients are maintained by a Na-K activated ATPase system both by electrophysiological and ultramicro-enzyme assay techniques.

Enzyme assays were carried out on the cochlear structures of the chicken and the guinea pig. After decapitation the cochlear structures were dissected, frozen on dry ice, lyophilized and stored at  $-25^{\circ}\text{C}$ . The frozen-dried structures were weighed on a Kahn electrobalance or on a quartz fiber balance (Lowry, 1953) and incubated in the substrate media as described by Bonting *et al.* (1963). The tissue concentrations varied between 0.2  $\mu\text{g}$  and 2  $\mu\text{g}/10\text{ ml}$  medium. Medium A (complete) gave total ATPase activity. The average activity of medium B (no K<sup>+</sup>) medium C (no Na<sup>+</sup>) medium D (medium A +  $10^{-4}\text{ M}$  ouabain) and medium E (medium B +  $10^{-4}\text{ M}$  ouabain) gave Mg-ATPase activity. Total ATPase activity minus Mg-ATPase activity gave the Na-K-ATPase activity. We also looked at the effect of K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> and ouabain on the Na-K ATPase activity by adding graded amounts of these substances to the media. The pH activity curves were obtained

TABLE 1 *ATPase activities in various cochlear structures*

Means with s.e.

Structure	Na-K ATPase		Mg-ATPase (moles/kg dr wt/hr)	Mean activity of turns nos.
	Moles/kg dr wt/hr	% of total ATPase		
Tegm. vasculosum	$5.10 \pm 0.96$	$30 \pm 3$	$8.10 \pm 1.10$	—
Stria vascularis	$7.95 \pm 0.43$	$50 \pm 1$	$5.57 \pm 0.41$	1, 2, 3
Lig. spirale				
(a) Behind stria vasc.	$0.40 \pm 0.09$	$53 \pm 7$	$0.36 \pm 0.09$	2, 3
(b) Comprising prom. spur and sulcus externus	$1.60 \pm 0.39$	$56 \pm 6$	$1.26 \pm 0.43$	2, 3
(c) Bordering scala tymp.	$0.35 \pm 0.07$	$35 \pm 7$	$0.63 \pm 0.07$	2, 3
Reissner's membrane	$0.33 \pm 0.06$	$38 \pm 4$	$0.91 \pm 0.10$	1, 2, 3, 4
Organ of Corti	$0.47 \pm 0.27$	$11 \pm 8$	$2.79 \pm 0.78$	2, 3

by incubation in media, prepared with buffers in the pH range from 6.2-9.6.

The role of Na-K ATPase in the generation of the cochlear potentials (cochlear microphonic potential and +endolymphatic potential) was determined by continuously perfusing the scala vestibuli of the guinea pig cochlea with Krebs Ringer solution during 45 minutes (10  $\mu$ l/min) with and without ouabain ( $10^{-6}$ – $10^{-4}$  M). In Table 1 the distribution of the Na-K-activated and Mg-activated ATPase activities in the cochlea is demonstrated. The stria vascularis and its homologous structure in the chicken, the tegmentum vasculosum, revealed a very high Na-K-ATPase activity. The Na-K-ATPase activity of Reissner's membrane, organ of Corti, and ligamentum spirale, except part b of this structure comprising promontoria spiralis and sulcus externus, is very low. Table 2 gives the activity of the stria vascularis in various turns. There is a decrease for both ATPase activities from the basal to the apical turn; this has also been demonstrated for

TABLE 2 *ATPase activity in various cochlear turns*

Means with s.e.

Turn no.	Na-K ATPase		Mg-ATPase (moles/kg dr wt/hr)	Total dry weight of stria etc., mg
	Moles/kg dr wt/hr	% of total ATPase		
1	$6.37 \pm 0.40$	$56 \pm 3$	$6.01 \pm 0.46$	13
2	$7.03 \pm 0.67$	$61 \pm 4$	$4.32 \pm 0.25$	12
3	$4.60 \pm 0.72$	$57 \pm 6$	$2.40 \pm 0.59$	7
4	$4.35 \pm 0.55$	$58 \pm 1$	$3.14 \pm 0.39$	3

## CATION TRANSPORT AND COCHLEAR FUNCTION

W. KUIJPERS

*From the Department of Otolaryngology University of Nijmegen Nijmegen  
Netherlands*

$\text{Na-K}$  activated ATPase was determined in the cochlear structures by ultramicro-analytical methods. The highest activity was found in the stria vascularis, with a decrease from base to apex. Ouabain, which inhibits the isolated enzyme system, appeared to diminish the  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  to the same extent. These findings strongly suggest that the  $\text{Na-K}$  ATPase enzyme system in the stria vascularis plays the key role in the maintenance of the cochlear cationic gradients, while such a role appears highly unlikely for Reissner's membrane. The  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  appear to depend on the functioning of this pump system.

As shown by many investigators, there is a characteristic difference between the ionic composition of the cochlear fluids (Fernández, 1967). The ionic composition of the perilymph is similar to that of other extracellular fluids. The endolymph—being an extracellular fluid—has an ionic composition similar to that of intracellular fluids.

It is now generally accepted that the maintenance of those cationic gradients in most animal cells depends on the activity of a  $\text{Na-K}$  activated ATPase that is specifically inhibited by cardiac glycosides (Skou, 1964).

In view of the cationic gradients in the cochlea between endo- and perilymph we have tried to determine whether these gradients are maintained by a  $\text{Na-K}$  activated ATPase system both by electrophysiological and ultramicro-enzyme assay techniques.

Enzyme assays were carried out on the cochlear structures of the chicken and the guinea pig. After decapitation the cochlear structures were dissected, frozen on dry ice, lyophilized and stored at  $-20^\circ\text{C}$ . The frozen-dried structures were weighed on a Kahn electrobalance or on a quartz fiber balance (Lowry, 1953) and incubated in the substrate media as described by Bonting *et al.* (1963). The tissue concentrations varied between 0.2  $\mu\text{g}$  and 2  $\mu\text{g}$  10  $\mu\text{l}$  medium. Medium A (complete) gave total ATPase activity. The average activity of medium B (no  $\text{K}^+$ ), medium C (no  $\text{Na}^+$ ), medium D (medium A +  $10^{-4}$  M ouabain) and medium E (medium B +  $10^{-4}$  M ouabain) gave  $\text{Mg-ATPase}$  activity. Total ATPase activity minus  $\text{Mg-ATPase}$  activity gave the  $\text{Na-K-ATPase}$  activity. We also looked at the effect of  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and ouabain on the  $\text{Na-K}$  ATPase activity by adding graded amounts of these substances to the media. The pH activity curves were obtained

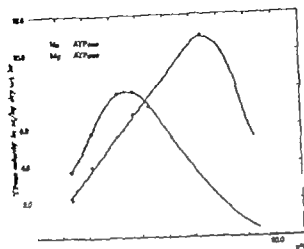


FIG. 2. Effect of pH on Na-K-ATPase (O—O) and Mg-ATPase (●—●) activities.

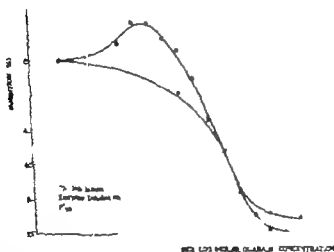


FIG. 3. Effect of ouabain on Na-K-ATPase activity (O—O) and on cochlear microphonic potential (●—●).  $pl_{50}$  against logarithm is the half maximal inhibition concentration.

of ouabain on the isolated enzyme system, also demonstrated in several other tissues (Bontling, 1966) could not be substantiated for CIMP.

In conclusion, it can be stated that the inner ear structures possess a Na-K-ATPase system that is similar to those described for other tissues. In view of its high Na-K-ATPase activity the stria vascularis and the tegmentum semicircularis are most likely the sites of the cationic pump, which regulates the composition of the endolymph. The role of the other cochlear structures in maintaining the cation gradients between endo- and peri-

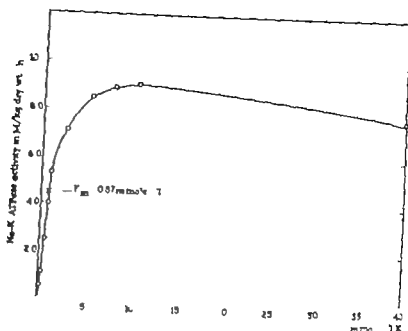


Fig. 1 Effect of K concentration on Na-K-ATPase activity

oxygen consumption (Meyer zum Gottesberge *et al.*, 1965) and for LDH and MDH (Kürschner 1968). Comparing the mean enzyme activity for the 1st, 2nd and 3rd turn of the totally dissected stria vascularis (Table 1) with that given in Table 2 for the same separate dissected turns, there is a difference of +22% taking into account the weight of the stria vascularis in these turns. Probably this is due to the time of dissection, which was for the whole cochlear stria quite shorter than for the stria vascularis of the separate turns. The decrease in activity from the 1st to the 4th turn cannot be influenced by this, because the apical turns were always first dissected. The activities for Reissner's membrane and Organ of Corti should also not be influenced by this effect, because these structures were dissected from cochleae that were lyophilized immediately after decapitation.

The activation of the Na-K-ATPase activity by K is given in Fig 1. Maximal activity was reached at 10 mM K, half maximal activation occurring at 0.8 mM. With Na, maximal Na-K-ATPase activity was reached at 10 mM Na and half maximal activation occurred at 4.5 mM. Maximal Na-K-ATPase activity was reached with 1-2 mM  $Mg^{2+}$  in the medium in the presence of 2 mM ATP. The pH activity curves are given in Fig 2. The optimum for Na-K-ATPase activity was pH 7.3 and for Mg-ATPase pH 8.7. Fig 3 represents the ouabain inhibition curve of the isolated enzyme system (open circles). The negative logarithm of the half maximal inhibition concentration was  $pI_{50} = 5.5$ . The effect of 40 minutes ouabain perfusion of the scala vestibuli on the CMP is also given in Fig 3 (filled circles). Comparing these two inhibition curves, there is a striking similarity in the effect of ouabain on the CMP and the isolated enzyme for both  $pI_{50} = 5.5$ . A similar effect was found for the +EI. The stimulating effect of low concentrations

- endolymphatic walls of the first turn of the guinea pig cochlea. *J Acoust Soc Am* 40 1232.
- KRECHENBERG, H. D. 1963 Über die Lactat- und Malatdehydrogenase in den vier Windungen der Cochlea des Meeresschnecken. Dissertation. Düsseldorf.
- LOWRY, O. H. 1952 The quantitative histochemistry of the brain, histological sampling. *J Histochem. Cytochem.* 1 420.
- MATTA VON GOTTENBERG, A., RAUCH, S., and KOSTER, E. 1965 Unterschied im Metabolismus der einzelnen Schneckenspiralwindungen. *Acta Otolaryng* (Stockh.) 59 116.
- RAUCH, S. 1964 *Biochemie des Hörsorgans*. Thieme Stuttgart.
- SACK, J. C. 1964 Enzymatic aspects of active Na<sup>+</sup> and K<sup>+</sup> transport through the cell membrane. *Prog. Biophys.* 13 133.

Dept. of Otolaryngology  
University of Nijmegen,  
Nijmegen, The Netherlands

### DISCUSSION

Ch. von Ilberg (1) Die höchste Aktivität von Na-K-aktivierter ATPase in Säugetiergeweben ist in Erythrozyten, Nerven und Gehirnzellen gemessen. Sie ist kein sicherer Indikator für Sekretion, sondern für einen aktiven Transport durch Zellmembranen. (2) Unsere eigenen Potentialmessungen (Ilberg u. Imamura Arch. Exp. Otol. Nas. K. h. h. p. 1966) zeigen durch Potentialmessungen an der isolierten Reissner-Membran einen aktiven Transport durch die Membran.

J. Angel-James It is surprising that the ATPase activity of Reissner's membrane is so low compared with that of the stria vascularis. Hughes & Chou measured the metabolic activity in several cochlear tissues by estimating the oxygen uptake.

They found a very high uptake in Reissner's membrane as well as in the stria vascularis, although the former was less than the latter. The activity was greater than that in renal tubules. With such a large differential in the electrolytes Na and K on the two sides of Reissner's membrane, which is composed of very few cells, we should expect a great metabolic activity to maintain this differential.

It is possible that the low ATPase activity observed was due to the very rapid post mortem changes which are known to take place. The specimens were taken after death and not under anaesthesia in the living animal.

Hughes & Chou showed that mixing of endolymph and perilymph due to enzyme inactivation takes place within half an hour after death.

W. K. Jaspers (Reply) to Ch. von Ilberg As far as I know the highest Na-K ATPase activity in mammalian cells is found in the kidney. The erythrocytes have a very low Na-K ATPase activity. The potential that you have measured on the isolated Reissner's membrane need not necessarily be the effect of an active transport mechanism. It may be the effect of passive diffusion.

T. Mr Angel-James At the congress on cochlear physiology in Halle this year Professor Rauch reported that few investigations by him on the oxygen consumption of Reissner's membrane did not confirm Dr Chou's earlier published results. The oxygen consumption appeared much lower. Concerning the loss of enzyme activity of the cochlear structures post mortem, I can say that the Na-K ATPase is a very stable enzyme system. In our experiments no significant loss of enzyme activity was found, keeping the isolated cochlear structures for more than 1 hour at 0°C.



lymph can be considered negligible. The assumption that transport of  $K$  from peri to endolymph would occur through Reissner's membrane (Rasch, 1964) must be highly unlikely in view of the low  $Na$ - $K$ -ATPase activity not only on a weight basis, but also on an absolute basis. The ratio of the dry weight of the stria vascularis and Reissner's membrane is approximately 1:1 this gives an  $Na$ - $K$ -ATPase ratio of 68:1. We may conclude, therefore, that the contribution of Reissner's membrane to active cation transport is very low. Additional arguments for this are the avascularity and the very high electrical resistance of this membrane (Johnstone *et al.*, 1966). This suggests that the  $K$  transport takes place through the spiral ligament and the stria vascularis.

The cochlear potentials, CMP and EP appear strongly dependent on the functioning of the  $Na$ - $K$ -ATPase system.

### RÉSUMÉ

L'enzyme  $Na$ - $K$ -ATPase a été déterminé par des méthodes ultra-microanalytiques dans la cochlée du cobaye. La plus grande activité de cet enzyme a été trouvée dans la strie vasculaire avec une diminution de l'activité de la base jusqu'à l'apex. L'ouabaine qui a un effet inhibiteur sur l'enzyme isolé porte le même effet sur le potentiel microphonique et le +EP. Ces observations portent à croire que le gradient de cations dans la cochlée formé par le  $Na$ - $K$ -ATPase est nécessaire à la fonction cochléaire.

### ZUSAMMENFASSUNG

In den Innenohrgeweben wird mit ultramikroanalytischen Methoden eine Ouabain-empfindliche  $Na$ - $K$ -aktivierte ATPase nachgewiesen. Die Aktivität war am höchsten in der Stria vascularis, mit einem Abfall von basal nach apikal. Die Potentiale CMP und +EP wurden mit Ouabain in gleichem Masse gehemmt wie das isolierte Enzym. Aus diesen Ergebnissen hat sich erwiesen, dass die  $Na$ - $K$ -aktivierte ATPase in der Stria vascularis die zentrale Rolle spielt zur Aufrechterhaltung der Kationengradienten im Innenohr, dass die Rolle der Reissner-Membran hierbei sehr gering ist und dass die CMP und +EP dem Funktionieren der  $Na$ - $K$ -ATPase Ionenpumpe abhängig sind.

### REFERENCES

- BORTING, S. L., 1966. Studies on  $Na$ - $K$ -activated adenosine triphosphatase. VI. The rectal gland of the Elasmobranchs. *Comp. Biochem. Physiol.* 17: 953.  
 BORTING, S. L., CARAVAGGIO, L. L., and H. WEISS, M. M., 1963. Studies on  $Na$ - $K$ -activated adenosine triphosphatase. VI. Its role in active transport in the renal cat, calf and rabbit. *Arch. Biochem. Biophys.* 101: 47.  
 FERNÁNDEZ, C., 1967. Biochemistry of laboratory fluids. *Arch. Otolaryng. (Chic.)* 88: 222.  
 JOHN STONE, B. M., JOHNSTON, J. R. and PROBERT, I. D., 1966. Membrane resistance in

- radioisotopic studies of the first turn of the guinea pig cochlea. *J Acoust Soc Am* 40 1393.
- KREMERER, H. ■ 1963 Charakteristika und Mechanismen der Elektrolyttransporte der Cochlea des Menschen. Dissertation. Düsseldorf.
- LOWRY, O. H., 1953 The quantitative histochemistry of the brain. *Histological* suppl. *J Histochem Cytochem* 1 320.
- MYER & GOTTSMAN, A., RICH, S. and KOWAN, E., 1965 Untersuchungen im Metabolismus der inneren Schwerkörnung. *Acta Otolaryng* (Stockh.) 59 118.
- RAUCH, S., 1964 *Biochemie d. Hörgangens*. Thieme, Stuttgart.
- SEAR, J. C., 1964 Enzymatic aspects of active linked transport of Na and K through the cell membrane. *Progr Biol* 16 123.

Dept. of Otolaryngology  
University of Nijmegen,  
Nijmegen & the Netherlands

## DISCUSSION

Ch. von Ilberg (1) Die höchste Aktivität von Na-K-aktivierter ATPase in Säugetiergeweben ist in Erythrozyten Nerven- und Gehirnzellen gemessen. Sie ist kein sicherer Indikator für Sekretion, sondern für einen aktiven Transport durch Zellmembranen. (2) Unsere eigenen Potentialmessungen (Ilberg u. Imamura Arch. Exp. Otol. Nas. Kehlkopf 1960) zeigen durch Potentialmessungen an der isolierten Reissner Membran einen aktiven Transport durch die Membran.

J. Angell James It is surprising that the ATPase activity in Reissner membrane is so low compared with that in the stria vascularis. Hughes & Chou measured the metabolic activity in several cochlear tissues by estimating the oxygen uptake.

They found very high uptake in Reissner's membrane as well as in the stria vascularis, although the former was less than the latter. The activity was greater than that in renal tubules. With such large differential in the electrolytes Na and K on the two sides of Reissner's membrane, which is composed of very few cells, we should expect great metabolic activity to maintain this differential.

It is possible that the low ATPase activity observed was due to the very rapid post mortem changes which are known to take place. The specimen were taken after death and not under anaesthesia in the living animal.

Hughes & Chou showed that mitling of endolymph and perilymph due to enzyme action takes place within half an hour after death.

W. K. Jpers (Reply) to Mr Ilberg As far as I know the highest Na-K ATPase activity in mammalian cells is found in the kidney. The erythrocytes have a very low Na-K-ATPase activity. The potential that you have measured on the isolated Reissner's membrane need not necessarily be the effect of an active transport mechanism. It may be the effect of passive diffusion.

T. Mr Angell James At the congress on cochlear physiology in Halle this year Professor Hauch reported that even in enligation by him on the O<sub>2</sub> consumption of Reissner's membrane did not confirm Dr Chou's earlier published results. The O<sub>2</sub> consumption appeared much lower. Concerning the loss of enzyme activity of the cochlear structures post mortem, I can say that the Na-K ATPase is a very stable enzyme system. In our experiments no significant loss of enzyme activity was found, keeping the isolated cochlear structures for more than 1 hour at 0°C.

## ISOENZYMES OF LACTIC DEHYDROGENASE OF THE PERILYMPH AND THE ENDOLYMPH

P. KLUYSKENS and W. VERSTRAETE

*From the E.N.T. Clinic The University of Ghent Ghent Belgium*

Enzyme dehydrogenase is directly related to carbohydrate metabolism. By histochemical methods LDH was found in some hair cells and the stria vascularis. Microchemical methods provided LDH iso-enzyme patterns in the endo- and perilymph.

Lactic dehydrogenase is a cytoplasmic enzyme which can be found in all human tissues. By histochemical methods, Spöndlin & Balogh (1963) have demonstrated that this enzyme is fairly concentrated in the sensory cells of the Corti's organ of the cat. Rauch (1964) has also found it in the vascular stria of the guinea pig. Lactic dehydrogenase catalyzes the reversible reaction lactic acid pyruvic acid. This fact is important in the intermediary metabolism of carbohydrates.

Lactic dehydrogenase like several other enzymes, can be present under different molecular forms which were named "isoenzymes" in 1939. Their negative electric charges are different and because of this fact they can be separated by electrophoresis. LDH1 has the most negative charge, LDH5 has practically no charge. LDH1 is prevalent in the oxidation of tissues where aerobic glycolysis dominates; on the other hand LDH5 is found particularly in tissues rich in lactic acid and anaerobic glycolysis.

Wieme (1959) showed not only that human tissues, but also rat and mouse tissues, contained the five fractions of LDH and that these fractions were also found in the serum. Since then, the dosage of these different fractions has influenced the diagnosis of certain illnesses such as infarctus, hepatic degeneration etc., each illness giving a different distribution of the various isoenzymes.

Silverstein & Schuknecht (1966) dosed total LDH of the perilymph in cases of otosclerosis, Menière's disease and acoustic tumours. They gave their results in international units. The total LDH value does not seem to produce any significant elements in the diagnosis of these diseases.

More recently Palva & Raunio (1967) have studied isoenzymes of LDH on the endolymph and perilymph of cadaver ears. Non-contaminated endolymph shows, in particular, fractions 1, 2 and 3, whereas in the perilymph fractions 4 and 5 are also observed. These two authors draw particular attention to contamination being a reason for errors.

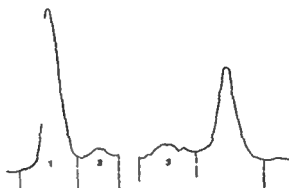


FIG. 1 LDH isoenzymes of the guinea pigs serum separated by agar-gel electrophoresis

Our first study of LDH isoenzymes was performed on endolymph and perilymph and compared to the LDH isoenzymes of serum in the guinea pig.

All contaminated samples were excluded. By means of marked capillaries, it was possible for us to examine the same quantity of liquid, i.e. 1 or 2. This quantity was sufficient to make a differential dosage of LDH on agar gel electrophoresis according to Wicme (1959). It seems superfluous to review this technique because of the full description given by the author.

The results obtained by electrophoresis of the serum in the guinea pigs showed that the fractions or isoenzymes of the LDH remained constant. The fraction LDH5 is too insignificant to be taken into account (Fig. 1). We would also like to draw attention to a second fact, that there was no important variation, which is also a point in favour of this method (Table 1) and confirms previous data.

We took samples of perilymph and endolymph in the same number of guinea pigs. Tables 2 and 3 summarize the results obtained. The important difference in the results for serum on the one hand and perilymph and endolymph on the other lies in the absence of an LDH4 fraction in the two latter liquids. If it existed, it was too minute to be shown.

TABLE 1 The LDH isoenzymes in serum of guinea pigs

Guinea pig no.	LDH1, %	LDH2, %	LDH3, %	LDH4, %
49	41.4	6.3	10.9	41.2
24	41.5	10.3	15.7	32.7
22	21.5	6.4	32.0	40.3
21	42.1	20.7	17.6	18.6
32	48.4	14.1	5.4	30.3
Average	39.5	11.8	11.3	32.7

TABLE 2 *The LDH isoenzymes in perilymph of guinea pigs*

Guinea pig no	Perilymph		
	LDH1 %	LDH2 %	LDH3 %
40	55.1	21.8	22.9
24	48.3	24.0	27.5
22	50.3	20.9	22.8
31	52.5	20.2	27.2
33	47.9	26.6	25.4
Average	52.4	22.7	25.16

The fact that no LDH4 fraction appeared in the perilymph and endolymph proves that aerobic glycolysis is the only important element for the metabolism of the inner ear. This fact is also proved by the much higher content of LDH1 and LDH2 in the perilymph and endolymph. In fact, we found 52.5% values in the endolymph and perilymph as opposed to 39% in the serum. The LDH2 content is double that of the serum. It is also important to draw attention to the fact that the isoenzymes are divided absolutely equally in the same concentration in the perilymph and the endolymph.

In the second part of our research, we have been able to show that the distribution of isoenzymes of LDH in humans with normal endolymph and perilymph is comparable to data traced in the guinea pigs. As we found in the guinea pig there is no LDH4 fraction in the normal perilymph and endolymph (Table 4).

We took samples of peri and endolymph in several cases of pathology of the inner ear or the ponto-cerebellar angle. Without venturing on a definite conclusion because of the small number of cases, we feel justified in drawing attention to certain significant facts taken from a diagnostic

TABLE 3 *The LDH isoenzymes in endolymph of guinea pigs*

Guinea pig no.	Endolymph		
	LDH1 %	LDH2 %	LDH3 %
49	48.9	23.4	27.8
4	53.2	23.1	23.7
22	53.2	21.7	22.1
31	59.7	23.3	16.9
33	51.0	24.0	25.0
Average	53.2	23.6	23.1

TABLE 4 Comparison between the different curves of isoenzymes of the serum of the perilymph and of the endolymph

	LDH1 %	LDH2, %	LDH3, %	LDH4 %
Human serum	47.4	23.1	13.1	16.4
Human perilymph	40.5	22.2	37.2	—
Human endolymph	39.2	36.2	22.8	—

TABLE 5. The LDH isoenzymes in normal human perilymph and in pathological conditions

	LDH1 %	LDH2, %	LDH3, %	LDH4 %
Normal human perilymph	40.5	22.2	37.2	—
Meningioma of the fovea posterior	33.4	27.0	18.6	20.8
Menière's disease	40.5	23.0	36.0	—
Cholesteatoma of the fovea posterior	33.7	33.1	19.1	13.8
Cerebellar tumour (astrocytoma)	43.1	37.7	19.1	—

point of view. These are given in Table 5. From this table it appears that in some cases of tumours in connection with perilymph circulation the LDH4 fraction is present.

These results justify a continuation of our research. In fact, there is clear evidence of the fraction LDH4 in certain tumours of the ponto-cerebellar angle. Perhaps if we continue our research it will be possible to use this new means for differential diagnosis between Menière's disease and tumours of the ponto-cerebellar angle.

### RÉSUMÉ

L'enzyme L.D.H. a un rôle important dans le métabolisme des hydrates de carbone. L'examen histologique a démontré sa présence au niveau de la strie vasculaire et des cellules sensorielles. Grâce aux techniques microchimiques, on peut doser L.D.H. dans le séro- et le périlymphe. Nous avons pu fractionner les isoenzymes de la L.D.H. en utilisant les techniques de fractionnement des protéines.

### ZUSAMMENFASSUNG

Die Dehydrogenase ist wesentlich für den Kohlenhydratmetabolismus. Mit Hilfe histochemischer Methoden wurde L.D.H. in Haarzelle und Stria vascularis dargestellt. Mit mikrochemischen- und analytischen Messverfahren konnten wir die Isoenzyme der L.D.H. in Endo- und Perilymphe dosieren.

## REFERENCES

- HOIDE, Y. YOSHIKAWA Y. and MORIMOTO M. 1962 Some oxidizing enzymes in the cochlea *Ann Otol* 71 96
- PALVA, T. and R. LUNO V., 1967 Disc electrophoretic studies of human perilymph and endolymph *Acta Otolaryng* (Stockh.) 63 123.
- RAUCH, S. 1964 *Biochimie des Hörgang* Thieme Stuttgart.
- SILVERSTEIN H. and SCHUKNECHT H. 1966 Biochemical studies of inner ear fluid in man *Arch Otolaryng* (Chic.) 81 305
- SPOKENDIX H. H. and BALOGH, K. 1963 Histochemical localization of dehydrogenases in the cochlea of living animal *Laryngoscope* 73 1061
- VOSTERN, K., 1960 The histochemistry of the enzymes of the oxygen metabolism in the inner ear *Laryngoscope* 70 351
- WIEBE, H. 1959 An improved technique of agar-gel electrophoresis on microscope slides *Clin Chim Acta* 4 317
- 1964 Diagnostic aspects of serum LDH isoenzymes. *Postgrad. Med* 35, A33.

E. V. Clinici University of Ghent  
De Pintelaan Ghent Belgium

## DISCUSSION

T. Palva I was very much interested in Mr Kluykens's paper because I have been working in the same field for a couple of years. His conclusions bring immediately to mind the studies of Silverstein & Schuknecht who found high perilymph total protein concentrations (average over 2100 mg%) in cases of acoustic neuroma. It may very well be that in future the use of microchemical methods will greatly increase our diagnostic possibilities, since taking perilymph samples is quite easy through the stapes footplate. LDH could very well be used in this respect too since we know that it appears also in cerebral extract, and that the cochlear aqueduct is capable of letting pass various substances from the cerebrospinal fluid space into the perilymph.

We have been able to show also small fractions of LDH and LDH<sub>2</sub> in the perilymph. This may be due to the fact that we have been using agarose and not agar which gives a poorer resolution of these isoenzymes. This was pointed out by Wijewe in 1968 and was confirmed in our studies.

Another factor that also has a bearing on LDH and LDH<sub>2</sub> findings is the storing of the fluids before the analysis is due. These isoenzymes may disappear if analyses are not made soon after the drawing of the specimens and I should like to know if there was any greater time delay before performance of the electrophoresis. Lastly the contamination of the samples is our present problem. The contamination with red cells which lack LDH is for this fraction negligible but a possibility with LDH. Has Mr Kluykens any idea how big a factor this might have been in his pathological cases?

P. Kluykens (Reply) to Mr Palva I do agree that the origin of the modification of the isoenzyme pattern may be located in the central nervous system. It has been demonstrated that the LDH and LDH<sub>2</sub> fractions are more evident when agarose is used. We only used the agar-gel electrophoresis on unstored endo- or perilymph.

In many cases the contamination by blood cells or serum cannot be avoided, but it is less when an electrocoagulation of the mucous membrane takes place before a small hole is bored in the footplate.

## THE TOTAL PROTEIN OF HUMAN PERILYMPH

T. PALVA and V. RAUNIO

*From the Department of Otolaryngology of University and from the State Sero-Bacteriological Laboratory, Oulu, Finland*

Total protein values were determined from the perilymph of 47 cadaver ears. After subtraction of hemoglobin the average total protein for two series of 27 and 10 ears was 553 and 559 mg% respectively. When the perilymph specimens were taken directly without freezing the average total protein figures were reduced to 280 (cochlea) or 312 mg% (vestibulum). The average hemoglobin concentration in the latter series was 42 and 39 mg% as compared with the values 134 and 118 mg% in the former two series. Albumin was the main constituent of perilymph proteins, accounting for more than half of the total. It is concluded that values representing the living normal state in man are difficult to obtain but, allowing for the post-mortem changes in CSF total protein, the true values may be estimated at 30 to 100 mg%.

Only a few analyses of the protein content in human perilymph have been made so far and the data reported are not uniform. Obviously this is due to the difficulty of obtaining human perilymph specimens during life without injuring the hearing and to the fact that the purity of the specimens leaves much to be desired.

In 1950 Waltner & Raymond reported on five cases of Menière's disease in which they compared the values for perilymph from the horizontal semicircular canal with those for the cerebrospinal fluid (CSF) using the ultraviolet absorption test. In conformity with visual estimation of perilymph, there was a strong peak at 410 millimicrons, testifying to the presence of hemoglobin; another peak was observed at 275 microns, end-absorption appearing at 265. Analyses of spinal fluid showed an absence of hemoglobin, low absorption at 275 micron with an endpoint at 265. Comparison of the absorption curves suggested that the protein content of perilymph is more than twice that of CSF.

In 1958, Ruch & Köstlin, without giving any details of their procedure, reported that the total protein content in human (post mortem) perilymph and endolymph is 70–100 mg% and 20–30 mg% respectively.

Hladky *et al.* (1960) obtained 26 perilymph specimens in otosclerosis and none in Menière's disease, reporting total protein values varying from 115 to 350 mg%. The samples were contaminated with blood up to 10% centri-

This study was supported by grant from the National Research Council for Medical Sciences.



fuge separation of erythrocytes generally yielded values close to the lower limit of the range given

Recently Schindler *et al* (1965) recorded an average value of  $728 \pm 463$  mg% for 13 perilymph samples, mainly from otosclerotic ears, with blood contamination of less than 35%. The amount of protein deriving from blood was estimated at 200 mg% which results in an average value 528 mg%. In a series reported by Rüedi *et al.*, consisting of 30 otosclerotic ears, the total protein value was  $965 \pm 430$  mg%. From this value the possible effect of mixed blood had already been subtracted.

Silverstein & Schuknecht (1960) reported the total protein as ranging from 150 to 770 mg% (mean 425) in 12 otosclerotic ears. In eight further cases with poor bone conduction the range was 130–825 mg% (mean 380 mg%). Similar values were found in nine cases of Menière's disease (range from 140 to 608 mg% mean 348). Widely differing results were found in seven cases of acoustic tumor here the range was 1400–3600 mg% and the mean as high as 2444. In a later series of 12 otosclerotic ears, the use of a meticulous technique to avoid blood contamination gave an average concentration of 195 mg% with a range of 89 to 326. In 10 normal ears studied  $1\frac{1}{2}$  to  $7\frac{1}{2}$  hours post mortem the average was 212 mg% (range 135–509).

Further results from Schuknecht's clinic were reported by Davies (1968). Within the limits of 1 to 24 hours post mortem there was no appreciable difference in the total protein figures, and a mean of 282 mg% (range 49–509) was obtained.

#### MATERIAL AND METHODS

Perilymph from 47 ears was collected post mortem using the methods described earlier (Palva & Raunio, 1967). After death the bodies were kept at +5°C until autopsied. The removed perilymph specimens were kept in a deep freezer until analyzed. Measurement of the total protein was performed by the method of Lowry *et al* (1951). An immunochemical method was employed for albumin determination (Mancini *et al.*, 1965). Hemoglobin content was determined on the basis of peroxidase activity of hemoglobin as suggested by Crosby & Furth (1956).

#### RESULTS

The first series of analyses were made on perilymph from 27 cadaver ears, generally removed 36 to 48 hours after death. The temporal bones were stored in a -40°C deep freezer until there were several pairs. Perilymph samples were taken after extraction of the stapes as soon as the cochlear fluids were defrozen at room temperature. The results are seen in Table 1. The total protein value was found to average 678 mg% whereas the average hemoglobin concentration was 124 mg%. When the individual hemoglobin values were subtracted from the initial total protein values, the figures shown in the third column were obtained. The final average was 554 mg%.

On reviewing these rather high results, it was decided to analyze a second

TABLE 1 *Perilymph protein analyses from 27 frozen specimens*

	Total protein	Hb	Total protein (Hb subtracted)
Mean	678	124	554
Range	460-1194	18-303	393-1076
s.d.	182	73	162

series in which the corresponding CSF values were included. The results for 10 such ears and CSF specimens are presented in Table 2. The perilymph analyses still yielded values similar to those in Table 1 (average 559 mg%) but the figures for CSF were all (with one exception) under 200 mg%. The extreme CSF value, 1166 mg% was found in a 58-year-old man who had died of coronary occlusion without signs of neurological disease. In this case as in all others, hemoglobin contamination of the CSF was very slight.

Following the discovery of this fairly great discrepancy between perilymph and CSF protein values, 10 further temporal bones were removed and perilymph collected immediately. Samples were taken first through the round window membrane and then from the vestibular labyrinth. The albumin content of each sample was also determined.

The results in Table 3 show that, after subtraction of the hemoglobin values, the remaining total protein from the scala tympani averaged 280 mg% and that from the vestibule 312 mg%. These values are not statistically different but both differ highly significantly from the values presented in Tables 1 and 2 ( $p < 0.0025$ ). The average albumin content of the perilymph

TABLE 2 *Perilymph and CSF protein analyses from frozen specimens*

Case no.	Perilymph			CSF		
	Total protein	Hb	Total protein (Hb subtracted)	Total protein	Hb	Total protein (Hb subtracted)
1. sin	878	131	745	147	0	147
2. sin	1200	40	1160	181	7	174
3. dr	878	295	583	150	0	150
sin	471	85	416			
4. sin	878	355	621	177	0	177
dr	389	51	338			
5. sin	715	113	602	1108	4	1161
dr	533	63	470			
6. dr	358	84	294	187	0	187
sin	478	117	361			
Mean	677	118	559	334	2	333
Range	358-1200	40-295	338-1160	147-1108	0-7	147-1161

TABLE 3 *Protein analyses on non frozen cochlear and vestibular perilymph*

Case no	Cochlear perilymph				Vestibular perilymph				CSF			
	Total protein	Total protein			Total protein	Total protein			Total protein	Total protein		
		Hb	(Hb subtracted)	Alb		Hb	(Hb subtracted)	Alb		Hb	(Hb subtracted)	Alb
1. dx	203	7	286	110	300	2	288	110	203	0	293	4
sin	16	3	243	110	293	0	293	111				
2. dx	328	5	323	—	338	4	334	250	680	4	676	110
sin	393	48	347	180	309	5	304	250				
3. dx	437	133	302	290	521	114	407	300	620	51	569	139
sin	419	113	306	180	414	48	366	270				
4. dx	250	30	220	45	347	65	282	150	139	8	131	0
sin	297	10	286	110	489	145	344	250				
6. dx	210	5	211	78	197	8	189	88	149	0	149	41
sin	330	58	281	96	246	2	244	96				
Mean	322	42	280	133	351	39	312	187	308	13	345	7
Range	216-437	3-133	211-347	78-290	197-521	0-145	180-407	88-300	139-680	0-51	131-676	0-110

samples from both sites accounts roughly for half of the total protein figure. The CSF total protein was much elevated in two cases (620 and 680 mg%) both were old people with pronounced atherosclerosis in the basal brain arteries but without any other central nervous system lesions. The hemoglobin was 51 mg% in one case and less than 10 mg% in all others.

### DISCUSSION

In these post mortem analyses, the factor apparently responsible for the high average values for total protein in Tables 1 and 2 was the process of freezing and defreezing before collection of the fluid specimens. In fact, perilymph was removed from two ears in icy crystals and the resulting figures were comparable to those in Table 3, thus storing of the bones as such did not increase the protein content. During the defreezing process both hemoglobin and plasma proteins may issue from the damaged capillaries into the perilymph space. This is apparent from Tables 1 to 3 which indicate that the hemoglobin concentration of perilymph from non-frozen specimens is only 40% of that in the frozen specimens.

It is interesting to note that our values from frozen specimens are of the same order as those obtained by Schludler *et al* (1985) and clearly lower than those of Rüedi *et al* (1965). This points to the same source of contamination, viz. erythrocytes and plasma which is inevitably present at routine stapedectomies. Thus the high figures for total protein in otosclerosis probably are artefact. Case 3 included in Table 3, showed otosclerosis in the right ear nevertheless, the value for total protein did not differ from

the other cases in any way. This is in conformity with the results of Hladky *et al* (1960) and Silverstein & Schuknecht (1966).

The values for perilymph, taken 11 to 72 hours post mortem (Table 3) coincide fairly well with those of Davies (mean 282 mg% range 49-503 mg%) whose 23 samples were all taken within 24 hours (six within two hours) although the hemoglobin concentration was not analyzed and subtracted. It is worth noticing that time within the limits 1-2 hours or 2-24 hours, had no effect upon the total protein values of Davies, and in our series they remained at the same level as late as 72 hours after death. Apparently the cessation of blood flow was responsible within the first hour for the post-mortem values, and these are not true values for living patients. This result is supported by the findings made by Rodgers & Chou (1966) and ourselves (Palva & Tikkanmäki, 1969) concerning post mortem  $\bar{M}$  and  $\bar{N}_a$  values, which were not found to change appreciably after the first few post-mortem hours.

Our figures for post mortem CSF total protein generally were below 200 mg% however there were three cases in which the values were much higher without apparent cause. The contamination of CSF with hemoglobin is generally low. Thus the artificial increase in the total protein content of post-mortem CSF specimens probably is lower than in perilymph.

Determination of albumin by the immunodiffusion method was carried out because we had earlier reached the conclusion (Palva & Raunio, 1967) that this protein is the main normal constituent of true perilymph proteins. The amount of albumin in the samples of this study (Table 3) concurs with this view.

Since both the post-mortem and intra-operation values do not represent the true status of human perilymph concentrations, one may truly wonder whether the real figures during life are known at all. As regards CSF there is no doubt the normal values vary between 15 and 30 mg% figures below 45-50 mg% still being normal. For perilymph, no investigators other than Rauch & Höslin have claimed to state normal values (between 70-100 mg%). Study of the Rauch-Höslin (1958) report, however gives no clues as to how the total protein figures were obtained. In Rauch's monography (1964) these figures were stated to be post mortem.

Nevertheless, when the general post mortem values of 150-200 mg% for CSF (Table 3) and the true normal figures of less than 50 mg% are considered, one cannot avoid the conclusion that a somewhat larger artificial increase to the perilymph value would reduce the average here obtained, 280-312 mg% to some 50-100 mg% in the normal living state. This, however remains speculative.

## RESUME

La teneur de la protéine totale de la périlymphe humaine a été déterminée dans 40 cas (post mortem). Les résultats sont influencés par conséquent, par les com-

posants du sérum On a déterminé aussi la protéine totale et l'hémoglobine du liquide céphalo-rachidien ainsi que la concentration d'albumine dans les deux liquides.

### ZUSAMMENFASSUNG

Die Gesamtproteine wurden von der Perilymphe in 47 Kadaver Ohren bestimmt. Nach der Subtraktion von Hämoglobin Werte waren die Gesamtproteine in zwei Serien von 27 und 10 Ohren 553 und 559 mg%. Direkte Proben ohne Gefrierung der Temporalknochen für Bewahrung gaben reduzierte Werte von 230 (Cochlea) oder 312 mg% (Vestibulum) für die Gesamtproteine. Die Mittelwerte für Hämoglobin in diesen Proben waren 42 und 39 mg% während die für die gefrorenen Spezimen 124 und 118 mg% waren. Albumin machte ungefähr 60% von den Gesamtproteinen aus. Die Schlussfolgerung ist, dass rechte Normalwerte für lebende normalhörige Menschen kaum zu finden sind gestützt bei den Analysen von post mortem Zerebrospinalflüssigkeit, diese sind wahrscheinlich von der Ordnung von 50 bis 100 mg%.

### REFERENCES

- CROSSBY W. H. and FLINT, F. W. 1956 Modification of benzidine method for measurement of hemoglobin in plasma and urine. *Biological* 11 360.
- DAVIES, D. G. 1968 Biochemistry of the inner ear fluids—experimental and clinical observations. *J. Laryng.* 8 301.
- HLADKY R., BRADA, Z. and KOCHET, A. 1960: Versuch inner blut mischen Biopsie der Perilymph bei perleren Kranken. *Acta Otolaryng. (Stockh.)* 51 424.
- LOWRY O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. 1951: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193 265.
- MANCINI, G., CARO, R. A. O. and HEREMANS, J. F. 1965 Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochimistry* 2 235.
- PALVA, T. and RAUNIO, V. 1967 Disc electrophoretic studies of human perilymph and endolymph. *Acta Otolaryng. (Stockh.)* 63 128.
- PALVA, T. and TIKANMÄKI, P.: Sodium and potassium concentrations in post mortem human labyrinthine fluids. *J. Laryng.* In press.
- RAUCH, S. 1964: *Biochemie des Hörgans Einführung in Methoden und Ergebnisse*. Thieme Stuttgart.
- RAUCH, S. and KÖSTLIN, A. 1968: Apect chimiques d'endolymphes et de l'perilymphes. *Pract. Otorhinolaryng. (Basel)* 20 287.
- RODGERS, K. and CHOU, J. T. F. 1966 Concentrations of inorganic ions in guinea-pig inner ear fluids. *J. Laryng.* 80 835.
- RÖDQVIST, L., SÄNG, M. C. and FISCHE, L. 1965: Untersuchung der Perilymphe nach Stapedektomie in Otosklerosefällen. *Acta Otolaryng. (Stockh.)* 59 239.
- SCHWOLEN, R., SCHNEIDER, E. A. and WULFSTADT, H. L. 1965 Comparative determinations of some electrolytes and organic substances in the perilymph of patients with otosclerosis. *Acta Otolaryng. (Stockh.)* 59 309.
- SILVERSTEIN, H. and SCHLANKMANT, H. F. 1966 Biochemical studies of inner ear fluid in man. *Arch. Otolaryng. (Chic.)* 83 395.
- WALTHER, J. G. and RAYMOND, S. 1950: On the chemical composition of the human perilymph and endolymph. *Laryngoscope* 60 912.

Dept. of Otolaryngology  
Oulu University Oulu  
Finland

## PRESBYCUSIS, PRESBYSTASIS AND PRESBYOSMIA AS CONSEQUENCES OF THE ANALOGOUS BIOLOGICAL PROCESS

J. KRMPOTIĆ NEMANIĆ

Zagreb Yugoslavia

We found in the basal coil of the spiral tractus and in the depth of the internal auditory meatus a reduction in the number of the openings through which the nerve fibres of the acoustic nerve pass. This reduction was due to the apposition of bony substance. On the sections through the acoustic nerve of the same subject, the number of fibres was also reduced in the corresponding area. Analogous changes were found in the region of vestibular areas and also in the posterior part of the cribriform plate of the ethmoidal bone. These three places are similar in shape, i.e. consisting of a system of small openings through which pass the nerve fibres. In all these three places the apposition of bony substance increases with age and in all these three organs the function diminishes with age. We believe, therefore, that apposition of bone causes compression and degeneration of nerve fibres and hence the senile changes in hearing, vestibular function, and smell.

It is well known that hearing diminishes in old age but it is not so clearly understood that the vestibular and olfactory functions are also reduced. We believe that these three physiological changes which develop progressively with aging are caused by an analogous biological process.

Impairment of hearing in advanced age, known by the name of primary idiopathic presbycusis, is a deafness of the perceptive type, without recruitment, which means that the changes are not primarily localised in the organ of Corti. It is characterised by a loss of hearing of high tones, so that the changes must be localised in the basal coil of the cochlea. The longest frequencies of 250-600 Hz, are preserved, while frequencies of 1000-2000 Hz are impaired much earlier. Frequencies of 4000-8000 Hz disappear as early as the 40th year.

Rossberg (1964) found on clinical material that the excitability of the vestibular system is also reduced with age, but this fact is not often discussed in the literature.

The conception of progressive senile anosmia is based on histological changes described by Překost (1958) but very little attention has been paid to this problem. Only few authors (Portmann *et al.* 1960; Hofmann, 1926; Vachide, 1903) mention it.

The reason why the changes on the cochlear apparatus have been investigated and discussed more than those on the vestibular and olfactory appa-

ratus lies in the fact that the changes in hearing become manifest sooner than those of the vestibular and olfactory apparatus. The vestibular apparatus has a greater possibility of compensation. Slight changes in the olfactory apparatus cannot easily be registered either by subjective or objective methods, so that they remain hidden for a long time. In the ear on the other hand the loss of some frequencies, especially in musically trained people, is very early registered by the patient himself and objective methods of examination.

The cochlear the vestibular nerve and the fila olfactoria belong to sensory nerves and they conduct irritations from the corresponding sensory organs. All these three nerves pass, on their way from the sensory epithelium to the central nervous system through a system of tiny holes in a thin bony plate. We found in the region of these tiny holes a progressive piling up of the bony substance from the fetal period to the end of life.

Examining a relatively large material of 100 skulls (200 pyramides) the fundus of the internal auditory meatus from the fetal period to 80 years of age under binocular lupe ( $\times 10$ ) we found that the number of holes in the spiral tract as well as in the vestibular areas diminishes progressively with age. The reduction of the number of holes was bigger in the basal turn than in the modiolar region. On cross-sections through the spiral tractus, we could establish that from the fetal period to the adult period the endosteal bone which forms the tractus increases in thickness by ten. Analogous thickening of the bony substance and reduction of the number of openings in the vestibular areas and saccular and utriculoampullar area were more evident when present because the number of holes, being smaller from the beginning could more easily be counted (Fig. 1). This piling up of the bony substance develops progressively.

At the end of the sixth or the beginning of the seventh fetal month, the openings in the spiral tractus are big and the bony substance delimiting them very scarce. By apposition of the bony substance in the region of the tractus, the holes become smaller and the bony substance between them thicker. This apposition is in the beginning more intensive in the region of the basal coil. In some cases the piling up of the bony substance is not uniform so that on some places there remain excavations in the bottom of which a certain number of holes for the nerve fibres can be seen. In fetuses the number of holes varies from 100-140 and in adults it diminishes by a third. As a result of the apposition of bony substance, we found in 13 specimens from the age 64-78 years in the region of the entrance of the cochlear branch of the auditory artery bony cuffs surrounding the artery. According to Schwartz the branches of the internal auditory artery enter the cochlea through the central canal and through one of the openings in the spiral tract. We found the cuffs in all mentioned cases only in the beginning of the basal coil except in one where there was at the same time also a cuff in the region of the central canal of the modiolus.



FIG. 1



FIG. 2

FIG. 1. A section through the acoustic nerve in the region of the tract spiralis (63-year-old person) (One can see the rarefaction of the nerve fibres in the basal coil in comparison with the modiolus region).

FIG. 2. Reduction of the holes in the vestibular area of the fundus.

The fact that the bony cuffs appear in the greatest number of cases in the basal coil is something that we must expect, because it is the place where the hyperostotic changes first develop, narrowing at the beginning and finally obliterating the holes for the fibres of the acoustic nerve, while the modiolus remains intact for a long time.

To be able to demonstrate the consequences of these changes in the fundus on the nerve fibres, we made histological sections through the acoustic nerve near the fundus, i.e. in the region where it imitates in shape the shape of the spiral tract.

The nerve is, in the region of fundus, divided into small bundles and has an envelope of connective tissue to a length of 2 mm. From that point centrally we found glial elements in the nerve and the bundles join to form a single nerve trunk.

On the nerve which we succeeded in cutting through in the region very close to the fundus in older people, where the holes of the tractus are reduced, we have seen that the number of nerve fibres in this region is also reduced (Fig. 2).

We did not count systematically the number of nerve fibres in the acoustic and vestibular nerve because we could, by the number we have counted, prove the findings of Rasmussen (1940). According to Rasmussen the number of cochlear fibres is reduced with age by more than half compared with the vestibular fibres. The absolute number of cochlear fibres is bigger than that of the vestibular fibres. The reduction of fibres is in general symmetrical as well as the reduction of the number of openings in the spiral tract.





FIG. 3 Reduction of the holes in the lamina cribrosa (66 years) compared with the case where the holes were wide and numerous (20 years)

Unfortunately we did not have the possibility of purchasing the audiogram of the specimens from which we examined the fundus and the nerve because these specimens were obtained during dissections in forensic medicine and pathological anatomy.

A completely analogous process was also found in a third region, where the nerve fibres also run through a system of tiny holes, i.e. in the region of the lamina cribrosa of the ethmoid. The number of holes is reduced here too in the *postero-anterior* direction by a thickening of the lamina cribrosa. This fact has already been described by Knäffel. We could prove this reduction on our material. We found cases with a complete disappearance of holes and nerve fibres in the posterior region of the cribrous plate. It was easier here to prove the reduction in a number of holes because the cribrous plate is more accessible than the fundus and the number of holes is not so big as in the spiral tract (Fig. 3).

Summarising all these findings, we came to the conclusion that all these three processes in old age are consequences of the piling up of the bony substance in the region of the nerve openings. This bony substance narrows the holes, forming tiny bony channels which are finally obliterated. The piling up of bony substance leads to compression of the nerve fibres and consequently to their atrophy followed by a loss of function.

We called by analogy with presbycusis, the changes of the vestibular and olfactory function due to aging *presbystasis* and *presbysmia*.

## RÉSUMÉ

Nous avons trouvé dans la région du crête spirale dans le conduit auditif interne une réduction du nombre des petits canaux par lesquels passent les fibres nerveuses du nerf acoustique. Cette réduction était due à une apposition osseuse. Sur les coupes à travers le nerf acoustique du même individu le nombre des fibres nerveuses était réduit aussi dans la région correspondante. Des changements analogues ont été trouvés aussi dans les régions vestibulaires, ainsi que dans la partie postérieure de la lame criblée de l'ethmoïde. Ces trois places sont très semblables en ce qui concerne le configuration, c'est à dire elles possèdent un système de petits canaux pour les fibres nerveuses. Dans ces trois places l'apposition osseuse accroît avec l'âge et la fonction de ces trois organes est réduite avec l'âge. Nous pensons qu'une apposition osseuse produit une compression et une dégénérescence des fibres nerveuses et par conséquent des changements seniles de l'ouïe, de l'appareil vestibulaire et de l'odorat.

## ZUSAMMENFASSUNG

Wir haben in dem der Basilarmembran entsprechenden Teil des Tractus spiralis im Boden des inneren Gehörganges eine Verminderung der Zahl der Öffnungen für die Nervenfasern des N. acusticus durch Auflagerung von Knochensubstanz gefunden. An den Schnitten durch den N. acusticus von demselben Individuum konnte man eine Verminderung der Nervenfasernzahl in der entsprechenden Gegend feststellen. Analoges Verhalten fanden wir in den vestibulären Arcen von der hinteren Partie der Lamina cribrosa des Ethmoidei. Diese drei Stellen sind ähnlich gebaut d.h. sie enthalten ein System von Öffnungen für den Durchtritt der Nervenfasern. In diesen drei Stellen vermehrt sich die Auflagerung von Knochensubstanz mit dem Alter und alle drei Organe sind durch eine partielle Abnutzung im Alter charakterisiert. Wir glauben deswegen, dass die Knochenauflagerung eine Kompression und Degeneration der Nervenfasern verursacht und somit die senilen Störungen des Gehörs, des Gleichgewichts und des Riechorgans.

## REFERENCES

- ALLEN, D. H. G. 1933 Makroskopische Anatomie der menschlichen A. u. E. des Gehörgangs. I. *Handbuch der Neurologie des Ohres*, I. Urban und Schwarzenberg, Berlin and Vienna.
- COHEN, C. B. 1932 The nerve acoustics. *Laryngoscope* 42, 2. (Symposium.)
- LOVELL, W. P. 1932 Histologic changes in the aging cochlea. *J. Gerontol.* 7: 172.
- DRUMMOND, H. A. E. 1946 Presbycusis. I. *Hals-, Nasen- und Ohrenheilkunde* (ed. J. Bertröder, R. Leuk, and F. Zollner) 3: 3. Thieme Stuttgart.
- FÄLTHAGEN, K. 1934 Histologische und anatomische Studien der Verabredigten Struktur und Funktion des Innenohrs. *Arch. Ohren- u. Halsheilk.* 170: 142.
- GUNN, H. H. 1932 Correlations of histologic observations and the acuity of hearing. *Acta Otolaryng.* (Stockh.) 17: 307.
- GULF, B. 1938 På synstämningens relation till hörselns förändring. *Slump Gerontol.* 3: 319.
- HORN, C. P. B. 1926 Der Geruchssinn beim Menschen. I. *Handbuch der normalen und pathologischen Physiologie* (ed. A. Bethe and O. von Bergmann) 3: 11. Berlin.

- HOLMEN, W., 1927 Gehörorgan In *Handbuch der mikroskopisch n Anatomie des Menschen* (ed W Möllendorf) 3 J Springer Berlin.
- KRMPOTIĆ, J., 1939 Données anatomiques indispensables à connaître pour une chirurgie effacee du nerf statoacoustique *Communication au Congrès de l' Soc Française d'Oto-Rhino-Laryngologie* (Octobre) 15
- 1963: Presbycusis und Presbyosmie als Folgen eines analogen biologischen Processes *Bull Soc Acad Jug* 8 5 135
- 1964 Presbyosia la, Abnahme der H aktionsgröße des Gleichgewichtsapparates mit fortschreitendem Lebensalter sowie die Ursache dieser Erscheinung *Bull Soc Acad Jug* 9 4 100
- LEDERER, L., 1928: Altersve Änderungen des Gehörorgan In *Handbuch d r Hals- Nasen- Ohrenheilkunde* (ed Denk r and Kahlers) 6 705
- MAYER, O 1919-1920 Das anatomische Substrat der Altersschwerhörigkeit. *Arch Ohr Nas Kehlkopfheilk* 105 1
- PHILIPPOV A J 1962 On the cause of presbycusis *J Laryng* 72 583.
- PORTMANN G et al 1960 *Otorhinolaryngologi* J I G Dolin, Paris.
- PRÉVOST J L., 1938 Mackerlinger W Die Schleimhaut der oberen Luftwege im Blickfeld neuerer Forschung *Arch Ohr Nas Kehlkopfheilk* 13 1 Verhandlungsbericht 80
- RAEVLASEK, A F., 1940 Studies of the VIIIth cranial nerve of man *Laryngoscope* 50 61
- ROSSAKHO, G 1964 Die Altersabhängigkeit der vestibulären Leistungsfähigkeit *Arch Ohr Nas Kehlkopfheilk* 181 475
- SAXÉN, A 1937 *Pathologie und Klinik der Altersschwerhörigkeit u w Acta Otolaryng* (Stockh) Suppl 23
- SCHUCKNECHT H F 1935 Presbycusis *Laryng scope* 85 402.
- SERGER, I and KRMPOTIĆ J 1938 Über die Ursache der progressiven Altersschwerhörigkeit (Presbycusis) *Acta Otolaryng* (Stockh) Suppl 113
- VASCHIDE, V 1903-1905 Recherches expérimentales sur l'olfaction des vieillards. *Compt rend Acad Sci* 13 827
- 1903 *Bull Laryng Ot Rhinol* 323

Dept / Anatomy  
Federala 8  
Zagreb Yugoslavia

## DISCUSSION

M Portmann: Félicitation pour cette magnifique collection des temporeaux. Cependant je ne suis pas tout à fait d'accord sur les conclusions très mécaniques avancées selon lesquelles c'est la compression qui est la cause primaire de l'atrophie du nerf. Los est le résultat d'un équilibre biologique très fin. Il est probable que Los ferme les espaces parce que le nerf a primitivement dégénéré et non l'inverse.

C S Hallpike: Do you think that the bony changes which you have observed might have a pathogenesis similar to those results shown by Mellanby from Vitamin A deprivation?

H H Vanmann: Frau Krmpotić sprach von der Schwierigkeit, die olfaktorische Leistung zu messen. Wir haben an der Berliner Klinik eine Apparatur entwickelt, mit welcher wir 3 Parameter für die Riechfunktion messen können: (1) bei Latenzzeit (bis zum Beginn der Riechensensation), (2) die relative Menge des angebotenen Riechstoffes und (3) des Luftvolumen, welches als Träger für die Riechsubstanz dient. — Wir haben Messungen an Serien von Versuchspersonen verschiedenen Alters bezüglich des Riechvermögens durchgeführt. Unsere Ergebnisse

besitzigen, dass beim alten Menschen die oben genannten Parameter signifikant schlechtere Ergebnisse geben als beim Jung n.

M. v. Eysck: Existe-t-il un parallélisme parfait entre le phénomène de fermeture des orif ces osseux et la quantité des cellules sensorielles dégénérées? A ez vous observé des cas où les cellules sont dégénérées alors qu'il n'y a pas d'occlusion des orif ces osseux?

A. Montand n: J'ai également admiré les beaux documents anatomiques de Mme Krmpotić. Vous avoiz fait une série d'examen vestibulométriques qui n'ont pas révélé de modification du seuil vestibulaire vraiment significative que l'on puisse comparer à une presbycusis. Aussi j'aimerais demander à Mme Krmpotić si elle ne pense pas que l'élément acoustique qu'elle a constaté joue u rôle plus important dans la pathogénie des troubles de presbystasis que les altérations osseuses?

H. Engström: It is very often difficult to state what the primary reason is for a modification of the anatomy as described here. Could it be, as we know from Bredberg's studies in the nose and Naesens studies of the olfactory region, that with increasing age a considerable number of sensory cells and nerve fibres—ganglion cells degenerate and for that reason fewer and smaller channels are needed in the bone. Could the narrowing thus be a consequence not the reason.

J. Krmpotić (Réponse): à Mr Portmann: C'est vrai qu'il est difficile quelque fois de dire ce qui est primaire et ce qui est secondaire mais nous avons l'impression d'après nos études sur un grand nombre des préparations de la période fétale jusqu'à l'âge avancé que l'opposition osseuse est primaire et la dégénération osseuse secondaire.

T. M. Uppik: We have not studied the relationship between the changes of the bone and cristallinosis, as yet. We believe that in case of presbycusis the bone does not play an important role in the changes of the bone because in the particular of vascular regions in contact with the liquor is not so close and the changes are analogous as in the cochlear region.

An H. Naumann: Ich gratuliere Hr. Naumann dass er die Möglichkeit hat die Riechmessungen so exakt durchzuführen wir haben leider nicht die dazu nötigen Geräte. Es freut mich aber dass er noch beiaktuell ein Nachteil des Riechvermögens mit dem Alter feststellen konnte.

T. M. Eysck: We are very sorry to say that for technical reason we did not have the possibility to follow the same subject the fate of the nerve cells, nerve fibres, and bone but we are doing our best to find a way to compare the audiogram before death with post-mortem findings of the bone organ of Corti, and nerve fibres.

A. Mr. Montand n: Le facteur vasculaire joue aussi un rôle mais selon nos études sur un grand nombre des temporaux nous avons l'impression que l'opposition osseuse est d'une importance prévalente.

T. M. Engström: Out of the previously mentioned reasons, we cannot say what happens with the ganglion cells and the hair cells. We did not have here the opportunity to examine in the same subject the ear and nose, so we cannot say anything about the parallelism of these processes of the same individual.

# THE INNERVATION OF THE DEVELOPING FOWL EMBRYO OTOCYST IN VIVO AND IN VITRO

I FRIEDMANN

*From the Department of Pathology The Institute of Laryngology and Otology  
University of London London England*

The development of the innervation of the sensory neuro-epithelium of the inner ear has been studied in organ cultures of the chicken-embryo otocyst and in the developing chicken embryo with particular reference to the origin of the nerve endings and neurones. It is suggested that they originate from their embryonic centres in the mesenchymatous tissue surrounding the otocyst and reach the neuro-epithelial cells through gaps in the otic capsule and the basement membrane.

The innervation of the inner ear has attracted much interest and one may recognise several phases in the progress of the neuro-histology of the inner ear roughly coinciding with the advances in our microscopical and histological techniques. Light microscopists such as Cajal, Held and others have laid the foundations and erected the building of neuro-histology; more recently, the electron microscopists have provided the internal decoration. Engstrom, Wersäll, Spoendlin, Catherine Smith and others have played an important pioneering role in this field.

Our studies on which the present paper is based have been carried out on tissue cultures of the isolated fowl embryo otocyst which have shown that the otocyst attains *in vitro* a high degree of differentiation at both the cellular and ultrastructural level although isolated from the central nervous system (Friedmann 1956, 1959, 1965, 1968, Friedmann & Bird 1961, 1967).

It is perhaps interesting to note that the technique of tissue culture was in fact born out of the great controversy between the supporters of the neurone or outgrowth theory among whom Cajal played a prominent role and of the supporters of Hensen's unitarian theory of the development of the nerve fibre.

Early in this century Ramon y Cajal's discoveries concerning the embryonic neurone were taken to a further stage by Ross Granville Harrison who in the first decade of this century was the first to demonstrate *in vitro* on a coverslip coated with clotted frog lymph that the nerve fibre developed from a nerve cell and to confirm experimentally the validity of the outgrowth theory (Harrison, 1907 and Hughes, 1968) this was formulated by Hiss, who in a paper on the developing human cord (Hiss, 1880) concluded that every nerve fibre originated from a single cell.

The views of Cajal, His and Harrison have by no means commanded a unanimous and final acceptance (Hughes, 1968). Hensen, the great opponent of the "outgrowth theory" maintained that the cells of a nerve center and its related end-organ arise from the division of a common parent cell and that the sensory nerve fibre between them originates from an initial connecting filament (Hensen, 1864).

As regards the innervation of the inner ear the outgrowth theory has again been challenged by Proctor & Proctor (1967) in an interesting paper "On the understanding of hereditary deafness". The authors claim to have shown in 71-hour fowl embryos "that the cells of the acoustic ganglion do indeed arise from the neuroepithelium of the otic vesicle. By 78 hours, the ganglion cells have formed from the wall of the otic vesicle. The innervation of the future sensory hair cells is accomplished. At 82 hours in the chick embryo the auditory ganglia migrate away from the auditory vesicle" (Proctor & Proctor 1967). These conclusions appear to be based on light microscopic evidence alone and on the description of a group of cells in the subepithelial tissues of the young otocyst considered to be neurones.

It has been our experience that in the early stages of development, the neurones are concentrated in the stato-acoustic ganglion or its "anlage" near the neural crest. We have in fact suggested that the explant should include some of these neurogenic elements so as to achieve complete differentiation and innervation of the sensory epithelium (Friedmann, 1956, 1965, 1968; Friedmann & Bird, 1961, 1967). Scattered neurones may be seen in the differentiated otocyst in the mesenchymatous tissue underneath the epithelium.

The present investigations have had the object further to clarify some of these important issues. Electron microscopic evidence will be presented that the large cells referred to by Proctor & Proctor are Schwann cells and often contain large fascicles of non-myelinated nerve axons which are derived from the neurones of the stato-acoustic ganglion. All the evidence to be described contradicts the suggestion that the cells of the acoustic ganglion could arise from the neuroepithelium of the otic vesicle and fully endorses the outgrowth theory of His (1868), Ramon y Cajal (1900) and Harrison (1907).

#### METHODS AND MATERIAL

The watchglass method of Fell & Gibson (1929) was used, as modified in our tissue culture laboratory. Over 6000 fowl embryo otocysts have been processed in 152 experiments (Friedmann & Bird, 1961; Friedmann, 1968). In the present experiments, 144 otocysts have been studied, and were grown both in air and in a controlled atmosphere of 9 per cent  $O_2$  and 5 per cent  $CO_2$  in a glass container. Eight, ten and twelve-day-old cultures were collected, fixed in 3 per cent buffered glutaraldehyde followed by osmic acid (Zetterqvist, 1956) and embedded in araldite. Sections were cut on an

L. K. B. III Ultratome an stained with uranyl acetate and with the simplified leadcitrate stain of Venable & Coggeshall (1965) and examined under a Siemens Elmiskop I

Embryo otocysts were obtained from fertilised hen-eggs incubated at 38 C. The incubated eggs were successively opened after 1, 2, 3 to 12 days incubation the embryos removed and the otocysts dissected and processed as above

### *Own Observations*

The otocyst of the 3 to 5 days fowl embryo, which served as the initial explant for tissue culture is lined by an undifferentiated epithelium which consists of several layers of cells. The surface layer is composed of tall cells the basal cells are cuboidal to polygonal in shape and rest on a basement membrane which may be absent in places. The indistinct cell membranes appear to have gaps and the epithelium has a syncytial appearance. Fig. 1 shows a 5-day-old embryo with similar features. Ribosomes and polyribosomes are abundant in the cytoplasm which contains a smaller number of mitochondria becoming more numerous as the otocyst matures. The Golgi apparatus is less well developed. The nuclei are large and the nucleoli prominent. There are usually numerous mitoses.

It is important to emphasise that there are no nerve fibres present at this early stage of development within the developing epithelium of the otocyst. Underneath the basement membrane there are large, oval cells which may contain occasional nerve axons, suggesting that these cells are of the Schwann type.

### *Development of the Embryonic Otocyst*

Around the 6th day of embryonic development the surface epithelium tends to become better organised and occasional rudimentary kinocilia may make their appearance.

There is now underneath the basement membrane, marked mitotic activity and large fascicles of non myelinated nerve fibres occupy the area (Fig. 2). Some of the large cells seen under the light microscope consist of Schwann cells associated with multiple nerve axons (Figs. 3 and 4). These structures may be single or may form groups which correspond to the groups of large cells seen underneath the basement membrane under the light, or phase contrast microscope.

From now on the innervation of the neuroepithelium proceeds at an obviously quickening tempo. In the embryonic otocyst of 8 to 10 days single nerve fibres or fascicles penetrate the basement membrane and become established between the basal or supporting cells. These cells seem to adopt the role of that of the Schwann cells, protecting and perhaps nourishing the multiple naked axons (Fig. 7). The nerves anchor themselves at the base of the sensory cells, where they form single or multiple nerve endings.



Fig. 10. 6-day-old embryo otolyst showing the surface epithelium with indistinct cell membranes. There is no obvious nerve tissue and no nerve endings within the surface epithelium in the early stages of development. Compare with Fig. 10. 14,000

(Fig. 10) Some of the nerve endings of the embryonic otolyst contain synaptic vesicles and synaptic structures, including terminal bars make their appearance.

The organelles of the cells have matured, and the Golgi apparatus is well





Fig. 2 Six-day-old embryo tissue stained with methyl green and large numbers of nerve axons  
in the subepithelial tissue 10,500

developed. On the surface of the cells ciliary structures, stereocilia and kinocilia have reached full differentiation.

### *Organ Cultures of the Otocyst*

The process just described in the chick embryo can be seen repeated in tissue cultures of the isolated fowl embryo otocyst. There appears to be a



FIG. 2. 36-hour-old embryo otocyst showing fascicles of loosely lined nerve on left; also with nucleus of Schwann cell. 7000.



FIG. 3. Embryo otocyst. Several Schwann cells associated with large myelinated nerve axons. 30,000.



Fig. 2 Six-day-old embryo otocyst with mitotic cell and large numbers of nerve cells in the subepithelial tissue. 10,500

developed. On the surface of the cells ciliary structures, stereocilia and kinocilia have reached full differentiation.

#### *Organ Cultures of the Otocyst*

The process just described in the chick embryo can be seen repeated in tissue cultures of the isolated fowl embryo otocyst. There appears to be a



FIG. 7. T. Chanda—old tissue culture of an isolated fowl embryo otocyst. Nucleus of non-myelinated nerves lying between the cell of the basal layer of the mesoepithelium of the differentiated fowl embryo otocyst. Note: axon penetrating the basement membrane. 14,000

delay of a day or two in comparison. In the fully differentiated cultures of a total age of 16 days there are beneath the basement membrane capillaries, large fibroblasts and large cartilage cells. There are also numerous myelinated and non-myelinated nerve fibres beneath the sensory areas and

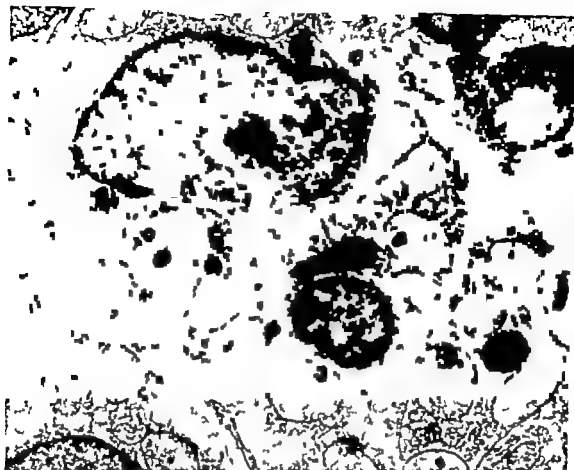


FIG. 5. Twelve-day-old tissue culture of an isolated fetal mouse embryo otocyst showing Schwann cells with multiple nerve axons forming large cell structures  $\times 28,000$ .

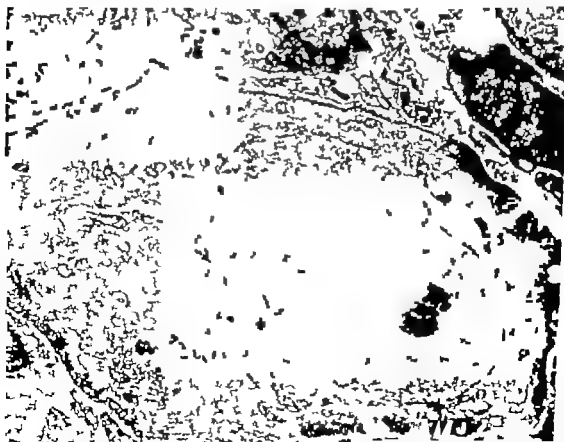


FIG. 6. Twelve-day-old tissue culture of isolated fetal mouse embryo otocyst. Gr. = ganglion; neuro = neuroepithelium.



FIG. 7. Ten-day-old tissue culture of an isolated fowl embryo otocyst. P axons of non-myelinated nerves lying between the cells of the basal layer of the neuroepithelium of the differentiated fowl embryo otocyst. Note nerve penetrating the basement membrane.  $\times 14,000$ .

day or two in comparison. In the fully differentiated cultures of a total age of 16 days there are beneath the basement membrane capillaries, large fibroblasts and large cartilage cells. There are also numerous myelinated and non-myelinated nerve fibres beneath the sensory areas and



Fig. 8. Twelve-day-old tissue culture of the isolated fowl embryo trophoblast. Multiple nerve endings at the base of a sensory hair cell. Sparse nuclei.  $\times 23,000$ .

multiple non myelinated nerve axons enveloped by one or more Schwann cells can be seen (Fig. 5). The nerve fibres penetrate the basement membrane singly or in fascicles, and losing their myelin sheaths, progress between the basal cells (Fig. 7). They ascend between the various cells of the neuroepithelium to reach the sensory cells where single bud-shaped nerve-endings or clusters of nerve endings are formed (Fig. 8). Cup-shaped



FIG. 8. Twelve-day-old tissue culture of salted fowl embryo otocyst. Synaptic region of hair cell which also contains terminal bar surrounded by vesicles. 52,500.

nerve ending or calyces may also be formed in the vestibular region (Friedmann & Bird, 1967). There are very few synaptic vesicles, although terminal bars surrounded by vesicles are frequent (Fig. 9). The calyces may form invagination filled with vesicles similar to synaptic vesicles. The surface of the sensory cell is by now fully equipped with stereocilia and kinocilia.





FIG. 10 Ten-day-old embryo (ocytan) cell with multiple nerve endings at the base of the cilia and organellum (compare with Fig. 1)  $\times 8000$

### Neurons

The neurons forming the stato-acoustic ganglion have characteristic electron microscopic features (Fig. 6) which differ from the ultrastructure of the large Schwann cells and similar structures observed underneath the

basement membrane and occur at some distance from the sensory epithelium.

Filamentous or granular neurones can be distinguished (Fig. 6). Some of the neurones seen formed a distinct axon hillock (Palay *et al.* 1963) from which the nerve fibres originate.

#### DISCUSSION

Fell (1929-1930) was first to succeed in the cultivation of the isolated fowl embryo otocyst and demonstrated differentiation of the hair cells and some of the other epithelial elements to Corti's organ *in vitro*. Fell was anxious to exclude any tissues outside the otic vesicle and her explants are a miracle of careful dissection. There is no mention of the development of any nerve tissue in her observations indicating that the absence of the primordial stato-acoustic ganglion in the original explanted otic vesicle prevented the development of nerve tissue in the neuroepithelium.

We have achieved complete differentiation *in vitro* of the sensory areas of the fowl embryo ear using a modified watchglass method. We have soon recognised that "the inclusion of certain neurogenic elements plays an essential role in the differentiation of the sensory areas" (Friedmann 1968). These are the stato-acoustic ganglion and eighth nerve primordia lying antero-ventrally near the neural crest and at some distance from the otic vesicle.

Proctor & Proctor (1967) have suggested that in the chick embryo the neurones of the acoustic ganglion developed from the neuroepithelial cells of the otocyst at about 71 hours and that following the establishment of the innervation of the sensory hair cells, at about 92 hours in the chick embryo, the auditory ganglion would migrate away from the otocyst.

This concept is based on light microscopic observations of the immature chick embryo otocyst. We have re-examined the ultrastructure of the embryonic otocyst and found no support for this theory. The large cells referred to by Proctor & Proctor are mostly Schwann cells. Moreover in the embryonic otocyst of 72 to 90 hours of embryonic age, there are no nerve axons within the developing epithelium.

The epithelial cells of the immature otocyst have no specific appearance and there are no nerve endings or synaptic structures present. These appear during the later stages of development of the neuroepithelium. There are no nerve fibres in the basal layers of the neuroepithelium either so prominent in the more advanced stages of development, when the number of the large Schwann cells has increased to form groups underneath the neuroepithelium. There may be found, in the differentiated otocyst occasional neurones among them. These however originate from the stato-acoustic ganglion and link up centrifugally with the neuroepithelium by means of outgrowing nerve axons. Terminal bars or deep invaginations surrounded by or filled with synaptic vesicles occur both in the 10-day old embryonic

otocyst and in the 12-day old cultures. These synaptic structures are comparable with the terminal bars described by Smith & Sjöstrand (1961) in the hair cells of the cochlea of the guinea pig who have suggested that they could play an important role in the function of the auditory nerve. None of these structures occur before the later stages of maturation and differentiation of the neuroepithelial cells and tissues.

Our observations lead logically to the conclusion that the innervation of the otocyst and *ceteris paribus*, of the inner ear develop centrifugally from the neurones of the stato-acoustic ganglia which are products of the neural crest. The neurones form axons which spread towards the neuroepithelium to reach the sensory cells, associated with Schwann cells, which, according to Weston (1963) also originate from neural crest material.

The outgrowth theory of course raises certain questions and one cannot but agree with Hughes (1968) lamenting that the outgrowth theory leaves unsolved the embryological problem of how it is that nerves connect with their right muscles and sense organs. Thus, we are left with the vexed problem of how nerve fibres of the eighth nerve find their way to their appropriate destination in the labyrinth of the inner ear.

## CONCLUSION

The development of the innervation of the neuroepithelium of the developing fowl embryo otocyst has been re-examined under the electron microscope based on a longstanding study of the embryonic otocyst and of organ cultures of the isolated fowl embryo otocyst *in vitro* which provided an excellent system for these investigations.

It has been shown that there are large Schwann cells, often enveloping large numbers of nerve axons in the subepithelial tissue which have to be distinguished from the neurones. It is pointed out that contrary to the suggestion of other authors there are no neurones in this area in the early stages of development of the otic vesicle. Moreover nerve axons, nerve endings and other synaptic structures are also absent within the neuroepithelium in embryos not older than 3 to 5 days.

As maturation and differentiation progresses, large fascicles of nerve axons reach the subepithelium from the more distant stato-acoustic ganglion and link up with Schwann cells. They proceed through the basement membrane and surrounded by various cells of the neuroepithelium, spread towards the sensory hair cells to form nerve endings and synaptic structures. The differentiation of the ciliary apparatus of the hair cells appears to be completed at about the same time 10 or 12 days in the embryo *in vivo* 12 to 14 days *in vitro*.

The evidence supports the outgrowth theory and disproves the theory that the cells of the acoustic ganglion arise through a division of the hair cells themselves.

## ACKNOWLEDGMENTS

My sincere thanks are due to my collaborator and chief technician E. S. Bird, F.R.S., to Mr David Eastham and Mr J. H. Connolly for the excellent illustrations and Mrs A. L. Thornton for secretarial help. The research has been aided by grants from the Central Research Fund of the University of London and the National Deaf Children's Society as well as the British Empire Cancer Campaign for Research.

## RESUME

Le développement de l'innervation du neuro-épithélium sensoriel de l'oreille interne et l'organe des terminaisons des nerfs et des neurones en particulier a été étudié au moyen de cultures d'organes de l'otocyste embryonnaire de la poule et de l'embryon développant du poulet. On suggère qu'elles prennent l'origine dans les centres embryonnaires dans le mésenchyme qui entoure l'otocyste et atteignent les cellules neuro-épithéliales par des trous dans la capsule optique et la membrane basilaire. Ce fait est en contradiction avec la théorie que les neurones du ganglion développent des cellules neuro-épithéliales et se dispersent dans les tissus environnants.

## ZUSAMMENFASSUNG

Die Entwicklung der Innervation des Labyrinths wurde am Organenkulturen des Otolysten und im Embryonalstudium, mit besonderer Rücksicht auf den Ursprung der Nervenendigungen und der Ganglienzellen. Es wird die Theorie vertreten, dass sie von den Anlagen im perilymphatischen Gewebe ihren Ursprung nehmen. Das widerspricht der Theorie, dass die Ganglienzellen des Innenohrs von den neuroepithelialen Zellen entspringen und in das umgebende Gewebe auswandern.

## REFERENCES

- CALL, RAMON, Y. 1960 *Studies on vertebrate neurogenesis* (translated by G. H. L.). Thomas, Springfield, Illinois.
- EMMONS, H. 1967 The ultrastructure of the sensory cells. *J. Laryng.* 81: 687.
- FALL, H. B. 1929-1930 The development in vitro of the isolated otocyst of the embryonic fowl. *Arch. Exp. Zellforsch.* 7: 89.
- FALL, H. B. and ROWEN, R. 1929 The growth and development and phosphatase activity of embryonic fowl otocysts and limb bud cultivated in vitro. *Biochem. J.* 23: 767.
- FALL, H. B. 1934 *In vitro culture of the isolated otocyst of the embryonic fowl. Ann. Otol.* 43: 93.
- 1939 Electron microscope observations on *in vitro* cultures of the isolated fowl embryonic otocyst. *J. Biophys. Biochem. Cytol.* 5: 263.
- 1963 *The Ear Cells and Tissues*. Culture edited by E. M. Whitter London and New York, 2: 321.
- 1968 The chick embryonic otocyst in tissue culture: model ear. *J. Laryng.* 82: 185.
- FALL, H. B. and DUNN, L. S. 1961 The effect of ototoxic antibiotics and of Penicillin on the sensory areas of the isolated fowl embryonic otocyst in organ culture. *J. Path. Bact.* 81: 81.
- 1967 Electron microscopic studies of the isolated fowl embryonic otocyst in tissue culture. Rodimentary kinocilia, cup-shaped neuroendings and synaptic bars. *J. Ultrastruct. R.* 16: 356.

- HARRISON R W 1907: Observation on the living developing nerve fibre *Anat Rec.* 1 116
- HELD H *Handbuch der normalen und pathologischen Physiologie* edited by A Bethe, C V Bergmann, G Emden and A Ellinger Berlin.
- HENRIE V 1864: Über die Entwicklung des Gewebes und der Nerve im Schwanz der Fische *Virchows Archiv* 31 51
- HISL W (cf Hughes, 1968) 1880: Anatomie menschlicher Embryonen.
- HUGHES A F W 1958 The growth of embryonic neurites. A study of cultures of chick neural tissues. *J Anat* 8 50
- 1968 *Aspects of Neural Ontogeny* Logos Press, Academic Press (London) Ltd.
- PALAT S L, SOTELO C, PETERS A and ORKAND, P H 1968 The organ hillock and the initial segment *J Cell Biol* 38 193
- PROCTOR, C. A and PROCTOR, B 1967 Understanding hereditary nerve deafness. *Arch Otolaryng* (Chic) 83 45
- SMITH, CATHERINE A 1967 Innervation of the cochlea *Rev Panam Otorinol Broncosofag* 1 1 2, 97
- SMITH, C. A and SJÖSTRAND F S. 1961 Structure of the nerve endings in the external hair cell of the guinea pig cochlea studied by serial sections *J Ultrastruct Res.* 523
- SPOE LUY H 1966 The organization of the cochlear receptor *Fortschr HNO-Ohrenheilk* 33 1
- VENABLES, J H and COGGESHALL, R 1966 A simplified lead citrate stain for use in electron microscopy *J Cell Biol* 5 407
- WERNILL, J 1936 Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig *Acta Otolaryng* (Stockh.) 5 ppl. 126
- WESTON J A 1963 A Radiographic Analysis of the Migration and Localization of Trunk Neural Crest Cells in the Chick *Devel Biol* 6 279
- ZETTERQVIST H 1936 *Histological Techniques for Electron Microscopy* Academic Press, London
- Dept of Pathology  
The Institute of Laryngology and Otolaryngology  
University of London  
330-332 Gage Inn Road London W.C.1  
England

## DISCUSSION

*J Wernill* The excellent studies by Mr Friedmann clearly demonstrated the importance of "model organs" which can be used for different studies in inner ear research. The otocyst has turned out to be an excellent organ for studies of early developmental morphology and I hope that somebody will soon use it also for physiological studies.

*H K Kristensen* As a supplement to the findings of Mr Friedmann I would like to mention our findings in a case of thalidomide intoxication

A girl aged 4 with no hearing had on tomography of the temporal bones no labyrinth. At autopsy no VIII nerves were found. On histological examination of the temporal bones there was on one side an empty cavity in the place of the labyrinth. On the other side there was also a cavity containing a well developed ampullary crest but no trace of nerve fibres below. This could not be interpreted until today with the results of Mr Friedmann's experiments.

*J Friedmann* (Reply) I thank Mr Wernill and Mr Kristensen for their encouraging remarks. Mr Kristensen's case seems to be an example of *in vivo* development of the inner ear in the absence of a central nervous link and control.

## INNERVATION PATTERNS IN THE ORGAN OF CORTI OF THE CAT

H. SPOENDLIN

*From the ENT Department of the University of Zürich, Zürich, Switzerland*

The afferent and efferent nerve supply of the organ of Corti was studied after transection of the olivocochlear fibres in the vestibular nerve, the vestibular root, or at the floor of the fourth ventricle. After elimination of the efferent fibres by degeneration, the distribution pattern of the afferent fibres were evaluated with electron and light-microscopy. In this study, all tunnel crossing radial fibres appeared to belong to the efferent innervation. The afferent fibres to the outer hair cell crossed the tunnel at the bottom basilar fibres. The majority of afferent neurons ended at the inner hair cells and only minority were associated with the outer hair cells. After selective transection of the crossed olivocochlear fibres, a great number of the efferent endings at the outer hair cells degenerated but also the number of internal spiral fibres seemed to be reduced.

During the last few years I have published some unexpected results on the innervation pattern of the cochlear receptor of the cat (Spoendlin, 1967 and 1968). These results have not been generally accepted, especially by electrophysiologists, who have expressed some doubt about the reliability of these anatomical observations (Eldredge, 1967). Because of the fundamental importance of such questions, a reinvestigation was essential to provide additional, incontestable evidence for those certainly unexpected, innervation features where for instance the majority of the cochlear neurons appear to be associated with the inner hair cells and only a minority with the outer hair cells.

A careful examination of the terminal nerve fibres in the organ of Corti, with evaluation of their relative numbers, is only possible at an ultrastructural level, since most of these unmyelinated fibres escape observation under the light microscope.

It is well known that the organ of Corti is not only provided with afferent nerve fibres, but also with a rather extensive efferent nerve supply through the olivocochlear bundle (Rasmussen, 1942). At the level of the modiolus, the efferent fibres are easily recognized as the intraganglionic spiral bundle (Fig. 1A) but within the organ of Corti both afferent and efferent fibres intermingled and can hardly be distinguished. However, their endings at the outer hair cell show clearly distinct features in electronmicroscopic preparations, the efferent endings being usually larger, filled with vesicles, and associated with a subsynaptic cisterna (Fig. 2).

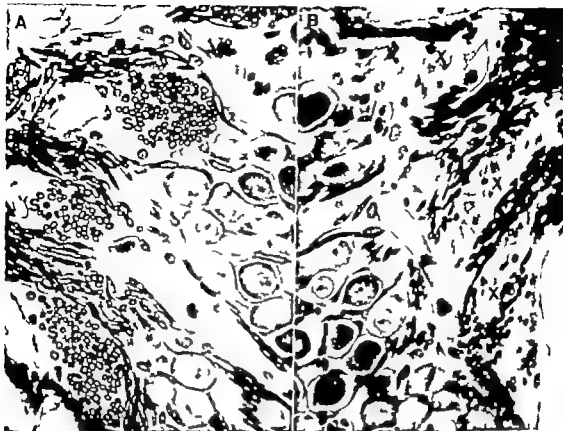


FIG. 1. Area of the intraganglionic spiral bundles of a cat in which the olivocochlear fibres have been transected on the right side 3 weeks previously by transection of the vestibular nerve. (A) Normal side with great bundle of numerous intraganglionic spiral fibres (S). (B) operated side. The intraganglionic spiral fibres are practically entirely missing (Y) and only Schwann cells can be seen in their location. There is an appreciable reduction of spiral ganglion cells.

In order to study the distribution pattern of the afferent nerve fibres within the organ of Corti, we induced degeneration of the efferent fibres by transection of the olivocochlear bundle in the vestibular root (Spoendlin & Gacek, 1963) or in the vestibular nerve (Spoendlin, 1967). Early signs of degeneration within the efferent endings in the form of concentric membranes around groups of mitochondria, were found 12 hours after the lesion (Fig. 3). After 2 weeks, degeneration was usually complete and, with the exception of occasional small cell debris, nerve endings and fibres had completely disappeared (Fig. 4). Judging from degeneration studies in the chinchilla by Smith & Rasmussen (1963) there might be considerable species-differences in the time course and mode of degeneration.

The fact that all richly vesiculated nerve endings at the outer hair cells, and most inner spiral fibres (Fig. 12) belong to the efferent innervation is, I think, today accepted by most people since at least in the cat they all degenerate after transection of the olivocochlear fibres. Subtotal degeneration of these endings was shown by Iurato (1962) in the rat and by Smith & Rasmussen in the chinchilla (1963) after lesions of the olivocochlear



FIG. 2. Nerve ending at the base of an outer hair cell (H) in normal animal. The efferent ending (N) is filled with synaptic vesicles and associated with subsynaptic cisterns on the hair cell membrane. The afferent terminals (A) contain only a few vesicles and appear to lack synaptic membranes. (Fixation with 1%  $\text{OsO}_4$  in phosphate buffer.)

fibres in the brain stem. However as we reported earlier, the tunnel crossing radial fibres also disappear after such lesions manifesting their efferent nature. The question whether all tunnel radial fibres are efferent or not is in fact of crucial importance for the study of the distribution of the afferent fibres. Therefore we will try to present as much evidence as possible that in the cat practically all tunnel radial fibres really belong to the olivo cochlear efferent system.

In experiment designed to provide such evidence two main possible errors have to be taken into account. The lesion of the olivo cochlear fibres must be complete and should not cause damage to the afferent cochlear



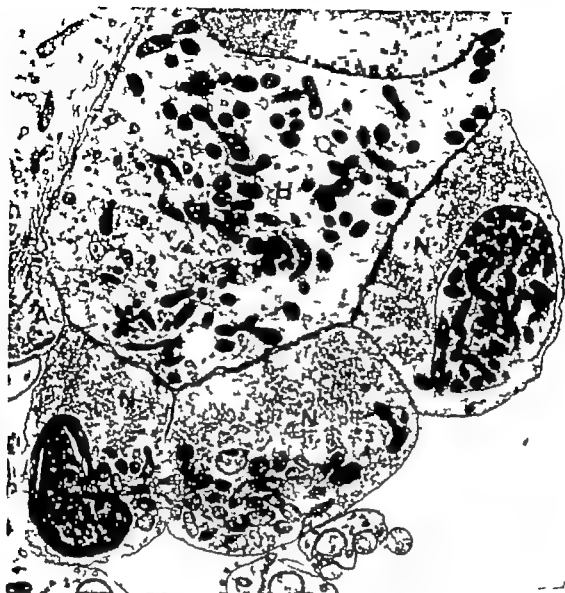


FIG. 3. Varicosity at the base of an olivocochlear cell (H) of a cat in which the contralateral olivocochlear fibres have been transected 18 hours previously by a midline lesion at the floor of the 4th ventricle. Already at this time signs of degeneration in the efferent nerve endings (V) are clearly seen in form of concentric membranes around groups of mitochondria. It is very marked in the lower left and in an initial state at the upper right of the picture. One afferent terminal is seen at (A). (Fixation with 2% OsO<sub>4</sub> in collidine buffer.)

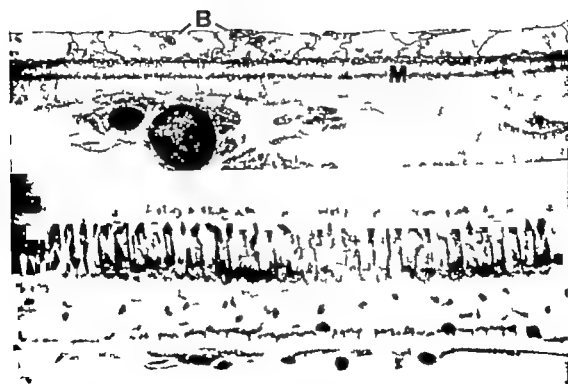
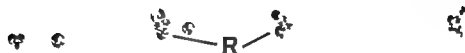
neurons. If the lesion is made by transection of the vestibular nerve in the internal acoustic meatus, all olivocochlear fibres are usually included—however, some afferent neurons can be damaged or the lesion may interfere with the cochlear blood supply. Such errors can be controlled by an evaluation of the number of spiral ganglion cells or the myelinated nerve fibres in the osseous spiral lamina. If these numbers are not appreciably reduced, important damage of the cochlear afferent neurons can be excluded. The efficiency of the lesion can also be checked by degeneration of the intra-



FIG. 4. Lower end of outer hair cell of cat 1 which the 1st cochlear fibres have been transected 3 days previous. The afferent nerve endings (A) are entirely degenerated and only some cytological debris are still present. The subsynaptic cisternae along the hair cell membrane appears to be normal. The afferent nerve terminals (A) have no clear structure.

ganglionic spiral fibres (Fig. 1B). Another cause of error is the difficulty of evaluating the totality of all tunnel radial fibres.

Different approaches were used to control these possible errors. The ears of one animal where the vestibular nerve was cut on one side 1 month previously were worked up in the way of normal temporal bone histology using celloidin embedding and 15-20 micron thick sections. Every section was carefully examined. In the normal control-ear 2-5 radial tunnel fibres were clearly seen in each organ of Corti of each section. In the operated ear they were missing and only one questionable remaining tunnel radial fibre was found throughout the entire cochlea. The intraganglionic spiral bundle was degenerated and the ganglion cell population in the spiral ganglion was



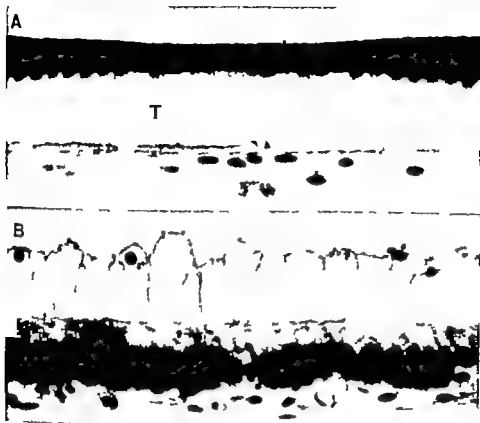
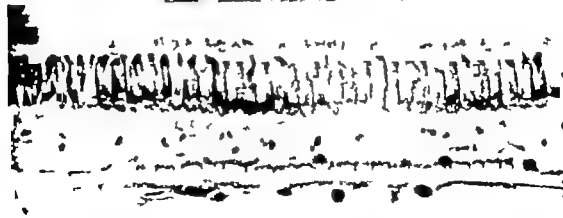
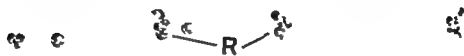


FIG. 6. (A) Light microscopic picture of tangential section through the tunnel of Corti in which the cochlear bundles have been transected in the calicula nervi 4 weeks previously. The tunnel space (T) appears entirely empty and no radial fibers are anymore seen. (B) Tangential section through the beginning of the osseous spiral lamina of the same ear as in section (A) (see schematic drawing) which shows that the number of the myelinated nerve fibres is not appreciably reduced.



FIG. 8. (A) Electron-micrograph of tangential section through the tunnel of Corti as indicated in the schematic drawing on the left. The fascicles of the tunnel crossing radial fibres (R) are transected and clearly seen how they lay entirely free in the tunnel. On the other hand there are the basilar fibres (B) at the bottom of the tunnel, most cases embedded in invaginations of the pillar cells. (B) Phase-contrast picture of a larger tangential section through the tunnel of Corti. Here, the fascicles of the tunnel radial fibres are still clearly visible as black spots.



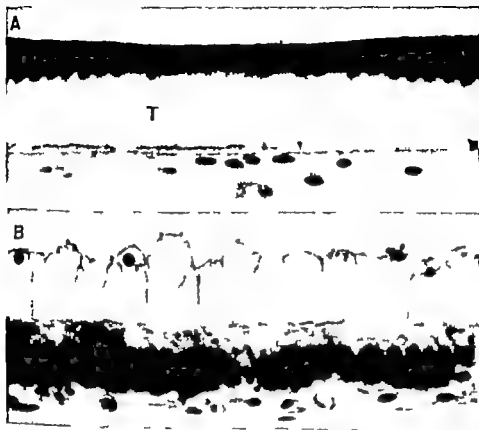


FIG. 8. (A) Light microscopie picture of tangential section through the tunnel of an animal in which the cochlear bundles have been transected 4 weeks previously. The tunnel space (T) appears entirely empty and no radial fibers are more seen. (B) Tangential section through the beginning of the osseous spiral lamina of the same area as in section A (see schematic drawing) which shows that the number of the innervated nerve fibers is not appreciably reduced.



FIG. 9. (A) Electron-micrograph of a sagittal section through the tunnel of Corti indicated in the schematic drawing on the left. The fascicles of the tunnel crossing radial fibers (R) are transversely sectioned and clearly seen how they lay entirely free of the tunnel. On the other hand there are the basilar fibers (B) at the bottom of the tunnel, most cases embedded in invaginations of the pillar cells. (B) Phase-contrast picture of a larger tangential section through the tunnel of Corti. Here the fascicles of the tunnel radial fibers are still clearly visible as black spots.

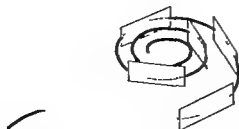


FIG. Schematic drawing showing how the tangential sections of the organ of Corti have been taken in order to evaluate as great portions of the organ of Corti as possible in phase contrast microscopy. In this way about  $\frac{1}{3}$  of the entire length of the organ of Corti of one cochlea can be examined.

normal showing an intact afferent nerve supply to the cochlea. In this way the entire cochlea could be examined, but the tunnel radial fibres are not very conspicuous in ordinary histological sections and an exact evaluation is hardly possible. With electron microscopy on the other hand, only very restricted areas of one cochlea can be examined.

Tangential sections through the tunnel of Corti where the tunnel is sectioned in a longitudinal direction, were found to be the best way to cover greater portions of the organ of Corti in one section and to visualize all tunnel radial fibres within the limits of each section. In these sections, the tunnel radial fibres appeared cross sectioned in fascicles of two to five fibres, presenting great variations in caliber. We measured diameters from 0.1 up to 0.6 microns (Figs 5 A, 8 A). In larger thicker sections for phase-contrast microscopy these small fascicles were still clearly visible as black spots and much larger areas of the organ of Corti could be examined (Fig 5 B). One month after transection of the olivo cochlear bundle these tunnel fibres had gone (Fig 6 A). Taking some sections through the osseous spiral lamina of exactly the same area, the condition of the afferent neurons was controlled and no signs of degeneration or reduction of their number could be seen (Fig 6 B).

From one cochlea several tangential sections could be obtained from the first and second turn which allowed us to cover about  $\frac{1}{3}$  of the entire cochlea (Fig 7). The cochleas of three animals with previous section of the olivo cochlear fibres were systematically examined in this way and in four other animals we evaluated one or two tangential sections of the basal turn. In all these examined specimens the tunnel radial fibres disappeared practically completely and there was no appreciable reduction of nerve fibres in the osseous spiral lamina, indicating an undamaged afferent nerve population.

Thus we confirm our earlier observations, that in the cat all tunnel radial fibres belong to the efferent olivo cochlear system.

The only remaining fibres crossing the tunnel are the basilar fibres which therefore have to be considered as afferent neurons (Figs 5 A, 12). The number of these basilar fibres reaching the area of the outer hair cells

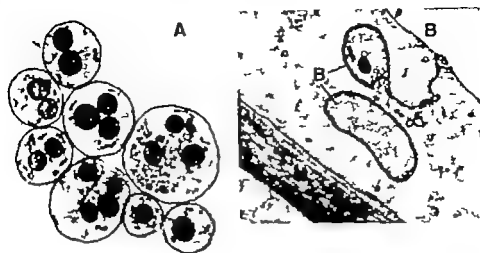


FIG. 8. (A) High magnification of tunnel radii fibres showing their large variation in diameter. (B) two basilar fibres (B) deeply embedded in deep invaginations of the pillar cell above the basilar membrane (M).

l surprisingly small. They can be counted easily as they penetrate between the outer or inner pillars. At the bottom of the tunnel they are usually embedded in invaginations of the pillar cell (Fig 8 B) and most of them take a short spiral course before they pass between the outer pillars. In tangential electron microscopic sections these small fibres could be seen cross-sectioned between the outer pillar feet (Fig. 9). In several sections of the first and second turn of the cochlea of different animals we evaluated the numbers of these fibres over a distance of approximately 1.5 mm, which correspond to about 200 outer pillars. We found a ratio of 186 nerve fibres to 200 outer pillars, which is an average of little less than one fibre per one pillar or a total of about 3000-5000 fibres. On the one hand, these relatively few basilar fibres appeared to be the only ones which were destined for the outer hair cells. On the other hand, practically all outer spiral fibres remained unchanged after elimination of the efferent nerve supply indicating that they belong to the afferent neuron (Fig 10). They are the continuation of the basilar fibres, which turn basalwards after having passed the outer pillars and run in a spiral direction over a considerable distance before they give off their terminal collaterals to several outer hair cells. The relation between the number of outer spiral fibres and basilar fibres penetrating between the outer pillars allows an estimation of the average spiral tension of the outer spiral fibres which is approximately 0.6 mm (Spennlin 1968).

Further evidence for such a predominant nerve supply to the inner hair cell was provided by reconstruction of the area between the habenula perforata and inner hair cell in animal where only the afferent dendrites are present the efferents being eliminated by previous transection and degenera-



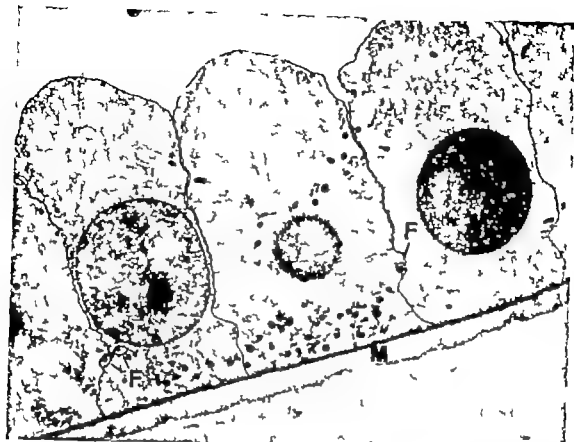


FIG. 9. Electron micrograph of a longitudinal section through the outer pillar cells as indicated in the schematic drawing. At this level the number of basal fibers (F) which penetrate between the outer pillars to reach the area of the outer hair cells can be counted. These fibers are not very regularly distributed. At some places two or three fibers run together at others only one fiber lies between two pillar cells and again at other places no fiber at all is found between two pillar cells. If such sections are evaluated over great distances we find an average of a little less than one afferent fibre between two pillar cells.



tion of the olivo cochlear fibres. In a series of several hundred sections, each individual fibre was followed from the habennula to its ending at the inner hair cell or to its passage between the inner pillars. For a better demonstration, we made a graphic reconstruction of the area of two inner hair cells on the basis of such serial sections. Six representative sections of this series were chosen to reconstruct the area in a drawing. The sectional plans used to build up this reconstruction are exact copies of the original electron micrographs from the series (Fig. 11). The fibres enter the organ of Corti in well packed bundles of about 10-20 fibres per habennular opening. These nerve fibre bundles are maintained for a short distance after which the fibres disperse between the supporting cells. The majority continue

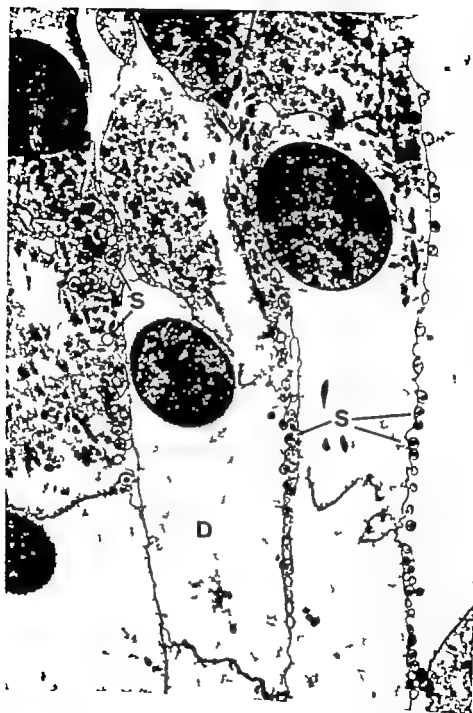


FIG. 10 Outer spiral fibers (S) between the three rows of Deiters cell (D) in animal where the II cochlear efferent fibers have been transected. The vestibular nerve 4 weeks post-operation. There is no perceptible reduction of the number of outer spiral fibers and this suggests that they are efferent nerve fibers.

in an essentially radial direction to reach the closest inner hair cell. Only very few fibres take a short spiral course before they penetrate between the inner pillars to continue as basilar fibres or before they end at one of the neighbouring inner hair cells. In reconstructions covering the area of five inner hair cells we found out of 20 afferent fibres entering the organ of Corti only one or two turning outwards to the outer hair cells. These numbers are consistent with the number of basilar fibres which pass between the outer pillars. The great majority of all fibres end unbranched with a single nerve ending at the inner hair cells. Each ending presents a typical synaptic complex, characterized by a marked thickening of the postsynaptic membrane, a minor accentuation of the presynaptic membrane, and a pronounced synaptic bar surrounded by vesicles within the sensory cell's cytoplasmic (cf. Smith & Sjöstrand, 1961).

All these observations give I think incontestable evidence that the great majority of sensory neurons are associated with the inner hair cells and only a minority with the outer hair cells, even though this might be against prevailing opinion.

If we count 50 000 cochlear neurons in the cat (Gaack & Rasmussen, 1961) only about 10% of them would be associated with the outer hair cells, where each fibre is connected with a considerable number of sensory cells. Kellerhals & Engström (1968) found among the spiral ganglion cells in guinea pigs approximately 10% ganglion cells which were different from the majority. Whether these correspond with neurons connected to the outer hair cells remains to be clarified.

The important functional significance of such a fibre distribution is evident. Considerations as to its possible functional implications are published elsewhere (Spoendlin 1967 and 1968). It could for instance provide a greater dynamic range for the organ of Corti, in such a way that the nerve fibres with multiple endings at the outer hair cells would be the most sensitive ones, whereas the fibres with single endings at the inner hair cells would have a higher threshold. Some sort of mechanism to increase the dynamic range of the cochlear receptor-organ has to be postulated because in single neurons the range of intensity between the first increase in rate of discharge up to the maximal rate of discharge is only about 30 dB, whereas the range between thresholds of the most sensitive fibre unit and the least sensitive unit at a given characteristic frequency may be 50 or 60 dB.

FIG. 11. Graphical reconstruction of the nerve of two inner hair cells in an animal in which the cochlear afferent fibres have been transected in the vestibular nerve 4 weeks prior to the reconstruction. The reconstruction made the basis of a series of several hundred sections. The sections represent serial sections as shown in the reference drawing. The section in plate 11 is the drawing of a reconstruction of the electron micrograph out of the serial drawing. On the electron micrograph shown a sample of the reconstruction of the section in plate 11 is shown. It is seen how the great majority of afferent dendrites is directed towards the inner hair cell and ends there with a single terminal.

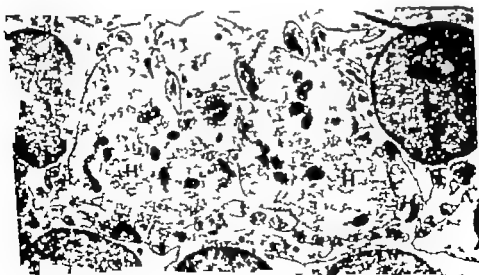
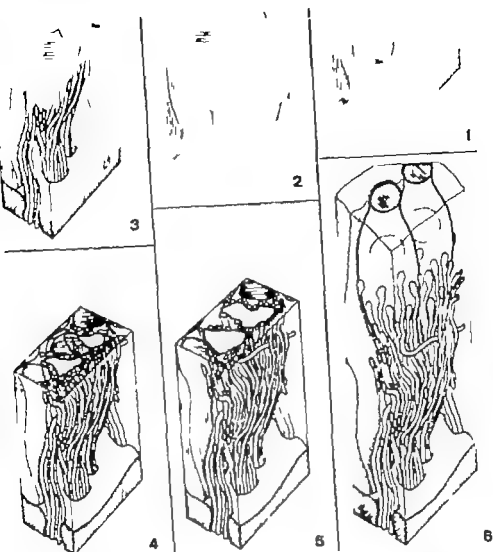




FIG. 12. Survey of the area below the internal hair cell (*H*). A bundle of nerve fibres emerges from the habenula opening (*O*). The majority of these fibres are afferent dendrites (*D*). Below the internal hair cell there are great numbers of very small inner spiral fibres (*S*). Adjacent to the inner pillar a similar group of nerve fibres forms the tunnel spiral bundle (*T*). At the lower left two basal fibres (*B*) are seen as they start their course across the floor of the tunnel. They appear to be the efferent dendrites for the outer hair cells.

(Davis, 1968). There seems, however, to be a gradual transition from the more sensitive neurons associated with the outer hair cells to the less sensitive neurons associated with the inner hair cells since Nelson Kiang (1965) in his studies on single primary auditory units, finds no outstanding group of especially sensitive fibres.

This innervation pattern, as it revealed itself in our anatomical studies, is not always in good agreement with electrophysiological observations. Thus Fex (1968) mentions two studies on single nerve fibres of the cochlear nerve of the cat where the activity of nearly all fibres could be inhibited

by stimulation of the crossed efferent fibres, which according to Iurato (1962) innervate only the outer hair cells in the rat. Here species-differences and the difficulty of evaluating the number of internal spiral fibres might play a role. In five cats where the crossed olivo-cochlear fibres have been cut by means of a midline lesion, we found the great majority of efferent fibres in the intraganglionic spiral bundle degenerated and we got the impression of a clear reduction of the internal spiral fibres. However an exact evaluation of these experiments will be published later.

## RÉSUMÉ

L'innervation efferente et afferente d'organe de Corti est étudiée dans le microscope électronique après lésion des nerfs suivants : des fibres olivo-cochléaires dans le nerf ou dans la racine vestibulaire du nerf cochléaire. Les résultats principaux portent sur la différence dans la distribution des fibres olivo-cochléaires croisées et non croisées et sur la distribution des neurones cochléaires dans l'organe de Corti, dont la majorité paraît d'être destinée pour les cellules ciliées internes.

## ZUSAMMENFASSUNG

Die afferente und die efferente Innervation des Cortischen Organes wird untersucht nach Durchtrennung der olivo-cochleären Fasern im Nervus vestibularis, in der Vestibularwurzel oder am Boden des vierten Ventrikels. Nach Elimination der efferenten Fasern durch Deafferentation kann das Verteilungsmuster der afferenten Fasern im Cortischen Organ mit Hilfe von Elektronen und Lichtmikroskopie untersucht werden. Bei der Katze scheinen alle zum efferierenden Radialfasern zur afferenten Innervation zu gehören. Die afferenten Fasern kreuzen den Tunnel im Boden des Vestibularfasern. Die grosse Mehrheit der afferenten Neuronen endigt an den inneren Haarzellen und nur ein kleiner Teil zieht zu den äusseren Haarzellen. Bei selektiver Durchtrennung der gekreuzten olivo-cochleären Fasern degeneriert eine Grosszahl der efferenten Endigungen an den äusseren Haarzellen, während die inneren Spiralfasern scheitern degeneriert zu werden.

## REFERENCES

- DIX, H. 1962 Discussion I. H. Spoendlin. In *Hearing mechanism and its disorders* (Ciba Symposium) p. 119. Churchill, London.
- ELIASON, D. H. 1967 Book review of H. Spoendlin. *The organization of the cochlear nerve*. *J. Acoust. Society* 41: 1394.
- FELT, J. 1968 Efferent inhibition in the cochlea by the olivo-cochlear bundle. I. *Hearing mechanism and its disorders* (Ciba Symposium) p. 168. Churchill, London.
- GAL, A. R. R. and R. W. C. L. 1963 Fiber analysis of the tectorial nerve. *J. Neurophysiol.* 26: 453.
- IURATO, A. 1962 Efferent fibers of the sensory cell of Corti organ. *Exp. Cell Res.* 27: 162.
- 1965 Personal communication.
- KATZ, A. H. and E. H. W. 1968 Die Morphologie des Ganglion spirale Cochleae. *Acta Otolaryng.* (Stockh.) Suppl. 226.



FIG. 12. Survey of the area below the internal hair cell (*H*). A bundle of nerve fibres merges from the habenula opening (*O*). The majority of these fibres are afferent dendrites (*D*). Below the internal hair cell there are great numbers of very small inner spiral fibres (*S*). Adjacent to the inner pillar is a similar group of nerve fibres forming the tunnel spiral bundle (*T*). At the lower left two basilar fibres (*B*) are seen as they start to cross across the floor of the tunnel. They appear to be the afferent dendrites of the outer hair cells.

(Davis, 1968). There seems, however, to be a gradual transition from the more sensitive neurons associated with the outer hair cells to the less sensitive neurons associated with the inner hair cells since Nelson Kiang (1965) in his studies on single primary auditory units, finds no outstanding group of especially sensitive fibres.

This innervation pattern, as it revealed itself in our anatomical studies, is not always in good agreement with electrophysiological observations. Thus Fex (1968) mentions two studies on single nerve fibres of the cochlear nerve of the cat where the activity of nearly all fibres could be inhibited

## COMPORTEMENT DE QUELQUES PHÉNOMÈNES AUDITIFS EN HYPOXIE

P. MENZIO

*Clinique ORL de l'Université de Turin Turin Italie*

L.A. fait des recherches sur le comportement de certains phénomènes auditifs en hypoxie (respiration pendant 5-10 min de mélanges au 7,5% et au 8,5% d'O<sub>2</sub> en %). Les tests ont été les suivants : sensation spatiale déterminée par deux tons d'égal intensité mais avec une petite différence de fréquence le test de discrimination dychoïque les tests vocaux avec distorsion ou accélérée et interrompue. L'hypoxie a déterminé des altérations particulièrement significatives pour les sensations spatiales et de moindre degré pour la voix distordue et accélérée. L.A. est de l'avis qu'une diminution de tension d'O<sub>2</sub> détermine de altération fonctionnelles surtout pour les procès qui se servent de circuits polysynaptiques et de intégration neurale précise.

On sait par une série de recherches que le flux circulatoire cérébral représente une entité relativement constante et qu'il ne ressent presque pas les variations de tension partielle de l'O<sub>2</sub>. Reich, par exemple, a démontré en 1964 que des changements contrôlés de la vitesse de flux hématique cérébral de 20 à 100 ml/min pour 100 gr de cerveau, produits par des variations contrôlés de la pression partielle de l'CO<sub>2</sub>, ne sont pas influencés par des changements de la tension partielle de l'O<sub>2</sub> hématique entre 60 et 380 mmHg. Un grand changement de pO<sub>2</sub> paraît ainsi compatible avec un flux hématique cérébral constant. Pour l'instant aussi la respiration d'O<sub>2</sub> pure cause seulement une petite diminution (13%) du flux hématique cérébral (Kely 1963).

On sait par contre qu'il y a un gradient très rapide de la tension partielle de l'O<sub>2</sub> entre les vaisseaux et les cellules cérébrales 33 mmHg dans les vaisseaux piaux et 1 mmHg au niveau intracellulaire pour Chance et autres. Des gradients similaires ont été démontrés par Mirsky entre les vaisseaux de l'épithélium et les cellules de l'organe de Corti.

Il faut enfin rappeler que la courbe d'utilisation de l'O<sub>2</sub> par les cellules augmente avec l'augmentation de l'activité fonctionnelle d'une façon non linéaire. (Dale & Crenell, 1962; Chance *et al* 1962) par un système enzymatique cellulaire très efficace qui permet une respiration cellulaire normale même avec une relativement basse tension d'O<sub>2</sub>.

Recherches analogues sur l'oreille interne ont été faites par Tunoo & Perlman (1963).



- KIANG, N Y-S 1965 Discharge patterns of single fibres in the cat's auditory nerve *MJT Res. Monograph*, 35
- RASMUSSEN, H L, 1942 An efferent cochlear bundle *Anat Rec* 82, 441
- SMITH, C. A., and RASMUSSEN, G L, 1963 Recent observations on the olivocochlear bundle *Ann Otol* 72 489
- SMITH, C. A. and SjöSTRAND, F 1961 A synaptic structure in the hair cells of the guinea pig cochlea *J Ultrastruct Res* 5 184
- SPOENDLIN, H 1967: The innervation of the organ of Corti, *J Laryng* 81 717
- 1968: Ultrastructure and peripheral innervation pattern of the receptor in relation to the first coding of the acoustic message. In *Hearing mechanism in vertebrates* (Ciba symposium) p 89 Churchill, London
- SPOENDLIN, H. and GACEK, R R 1963 Electronmicroscope study of the efferent and afferent innervation of the organ of Corti in the cat. *Ann Otol* 72 1

*ENT Department the University of  
Zürich Zürich Switzerland*

## DISCUSSION

*H Engström* If as there is reason to believe, Mr Spoendlin's observations are correct, and if there is no factor involved that confuses the pattern of degeneration I do believe that his findings are of extreme importance for the understanding of the cochlear function. We have mainly used guinea pigs and monkeys in our studies and it is clear that these animals differ from the cat, but I cannot say yet if the same principle of extremely rich innervation of the inner hair cells is present or not. Certain information from humans indicates that if inner hair cells are damaged this has greater importance for the degeneration of the nerve fibres in the spiral osseous lamina than a comparable degeneration of the outer hair cells. This would be in good agreement with Spoendlin's findings.

*A Meyer am Gottesberge* Die eindrucksvollen Bilder von Hr Spoendlin zeigen die dichte Innervation des inneren Haarzellen. Könnte es sein, wie ich 1948 vorschlug, dass das Recruitment davon abhängt, dass die äusseren Haarzellen abgenerieren die inneren jedoch erhalten sind und infolge ihrer höheren Schwelle und ihrer dichten Innervation den Lautheitsanstieg beziehen?

*H Spoendlin (Reply)* to Mr Engström: I am fully aware of the fact that there are considerable differences between animal species. In guinea pigs, for instance there are many afferent fibres crossing the tunnel at a higher level. The separation of afferent and efferent fibres is especially clear in the cat, which was one of the reasons why I chose this animal. I tried to get as much evidence as possible for these innervation-patterns and I hope that now others are repeating similar experiments to reinforce the evidence.

To Mr Meyer am Gottesberge: Our findings would in fact support the view that recruitment occurs when the outer hair cells are missing. The extensive ramifications of the afferent fibres at the outer hair cells, where one fibre innervates a great number of outer hair cells, and the observation that each inner hair cell is innervated by a great number of unramified afferent neurons, suggests a higher threshold for the activation of the nerve fibres at the inner hair cells. Once this threshold is reached however a great number of fibres are probably activated together.

TABLEAU 3. Résultats obtenus pendant la respiration avec un mélange pauvre en oxygène (8,6% d'O<sub>2</sub>)

Fréquence	Type	Après 2 min res	Après 5 minutes
125	1-3	5-0	3-1
250	2	5-0	3-1
500	3-4	0	4-5-0

conditions optimales on a la sensation d'un déplacement de la source sonore tout autour de la tête. Autres fois le déplacement subjectif est limité au devant, au derrière ou au dessus de la tête. nous pouvons enfin avoir une sensation de déplacement limitée à des lés ou des flots alternativement à droite et à gauche de la tête ou finalement on peut avoir simplement une vague sensation de déplacement sans en préciser la localisation (Tableau 2).

Les tests susmentionnés ont été faits sur des sujets de moins de 35 ans, sains, avec fonction auditive normale. Après les contrôles préliminaires les sujets respiraient des mélanges pauvres en O<sub>2</sub> (respectivement 8,6% et 7,6% d'O<sub>2</sub> en %). Les déterminations étaient faites préalablement, après 2 et après 5 minutes de respiration du mélange. Les résultats sont indiquées sur les Tableaux 3 et 4 et sont exprimés par les chiffres qui caractérisent les différents types de sensation sur la Tableau 2.

Si on analyse ces résultats on voit après 2 min la disparition des sensations spatiales plus fines (comme ce déplacement du son autour de la tête ou bien au devant, au derrière ou au dessus de la tête). Les sujets étudiés n'ont pas de sensation de mouvement du son dans l'espace ou bien ils ont seulement une sensation de déplacement de la source sonore incomplète et vague. Après 5 min de respiration des mélanges pauvres en O<sub>2</sub> on a une certaine reprise des sensations spatiales, tout de même sans rejoindre les valeurs normales. La reprise de sensation est plus évidente avec la respiration du mélange au 8,6% d'O<sub>2</sub> plutôt qu'avec le mélange au 7,6% d'O<sub>2</sub>.

Une deuxième série d'observations a été faite avec l'emploi du test de discrimination dychotique qui est constitué par une double série de mots et de chiffres entre eux différents, qui sont envoyés séparément et au même

TABLEAU 4. Résultats obtenus pendant la respiration avec un mélange pauvre en oxygène (7,6% d'O<sub>2</sub>)

Fréquence	Type	Après 2 minutes	Après 5 minutes
125	1-2	3-0	4-3
250	2	4-0	4-3
500	3-4	0	3-0

TABLEAU 1 *Caractéristiques des ondes employées*

I dB	F	F	$\Delta f/f$ %	$\lambda_0$
40	125	125.4	0,32	1/3
40	250	250.4	0.16	1/3
40	500	500.4	0,08	1/3'
40	1000	1000.4	0,04	1/3

Cependant il y a des conditions où la réduction de tension de  $O_2$  au dessous d'un certain niveau critique provoque une diminution de la respiration cellulaire. Les altérations fonctionnelles qui en suivent sont particulièrement évidentes pour ces phénomènes qui se réalisent par des circuits polineuronaux et qui demandent une intégration des activités neurales.

A la Clinique ORL de l'Université de Turin nous sommes en train de faire une série de recherches par différentes méthodiques sur le comportement de quelques phénomènes qui intéressent la voie acoustique centrale. Nous avons étudié les altérations induites par l'Hypoxie sur certains tests et nous avons recherché si il y a des rapports avec les résultats observés chez des sujets avec des altérations du système nerveux central.

Un premier groupe de recherches a été fait sur l'ainsi dite sensation spatiale acoustique déterminée par deux sons qui agissent sur les deux oreilles avec égale intensité (40 dB au dessus du seuil) et une très petite différence de fréquence (moins du 0.5%). On obtient ainsi une période de modulation constante et inférieure à la minute seconde. Les fréquences employées ont été de 125 250 500 et 1000 Hz (Tableau 1). Des recherches préliminaires faites par Menzio et Schindler sur des jeunes auditivement normaux ont démontré qu'on peut réaliser des différentes sensations spatiales plus ou moins nettes selon les sujets et les bandes de fréquence employées. En

TABLEAU 2 *Types de sensation*





1		Sensation spatiale complète à tour de la tête
2		Sensation spatiale complète Au devant Au derrière Au dessus de la tête
3		Sensation spatiale incomplète (grosses îles droite et gauche)
4		Sensation spatiale incomplète (îlots droite et gauche)
5		Sensation spatiale incomplète Pas de localisation

TABLEAU 3 Résultats obtenus pendant la respiration avec un mélange pauvre en oxygène (8,6% d'O)

Fréquence	Type	Après 2 minutes	Après 5 minutes
125	1-2	5-0	3-1
250	2	5-0	3-1
500	3-4	0	4-5-0

conditions optimales on a la sensation d'un déplacement de la source sonore tout autour de la tête. Autres fois le déplacement subjectif est limité au devant, au derrière ou au dessus de la tête nous pouvons enfin avoir une sensation de déplacement limitée à des lies ou des liots alternativement à droite et à gauche de la tête ou finalement on peut avoir simplement une vague sensation de déplacement sans en préciser la localisation (Tableau 2).

Les tests summentionnés ont été faits sur des sujets de moins de 35 ans, sains, avec fonction auditive normale. Après les contrôles préliminaires les sujets respiraient des mélanges pauvres en O<sub>2</sub> (respectivement 8,6% et 7,6% d'O en N). Les déterminations étaient faites préalablement, après 2 et après 5 minutes de respiration du mélange. Les résultats sont indiquées sur les Tableaux 3 et 4 et sont exprimés par les chiffres qui caractérisent les différents types de sensation sur la Tableau 2.

Si on analyse ces résultats on voit après 2 min la disparition des sensations spatiales plus fines (comme ce déplacement du son autour de la tête, ou bien au devant, au derrière ou au dessus de la tête). Les sujets étudiés n'ont pas de sensation de mouvement du son dans l'espace ou bien ils ont seulement une sensation de déplacement de la source sonore incomplète et vague. Après 5 min de respiration des mélanges pauvres en O<sub>2</sub> on a une certaine reprise des sensations spatiales, tout de même sans rejoindre les valeurs normales. La reprise de sensation est plus évidente avec la respiration du mélange au 8,6% d'O plutôt qu'avec le mélange au 7,6% d'O<sub>2</sub>.

Une deuxième série d'observations a été faite avec l'emploi du test de discrimination dychotique qui est constitué par une double série de mots et de chiffres entr'eux différents, qui sont envoyés séparément et au même

TABLEAU 4 Résultats obtenus pendant la respiration avec un mélange pauvre en oxygène (7,6% d'O)

Fréquence	Type	Après 2 minutes	Après 5 minutes
125	1-2	5-0	4-5
250	2	5-0	4-5
500	3-4	0	5-0

TABLEAU 1 *Caractéristiques des ondes employées*





I dB	F	F	$\Delta f/f$ %	$\lambda_0$
40	125	125,4	0,32	1/5
40	250	250,4	0,16	1/5
40	500	500,4	0,08	1/5
40	1000	1000,4	0,04	1/5

Cependant il y a des conditions où la réduction de tension de O au dessous d'un certain niveau critique provoque une diminution de la respiration cellulaire. Les altérations fonctionnelles qui en suivent sont particulièrement évidentes pour ces phénomènes qui se réalisent par des circuits polineuronaux et qui demandent une intégration des activités neurales.

A la Clinique ORL de l'Université de Turin nous sommes en train de faire une série de recherches par différentes méthodiques sur le comportement de quelques phénomènes qui intéressent la voie acoustique centrale. Nous avons étudié les altérations induites par l'Hypoxie sur certains tests et nous avons recherché si il y a des rapports avec les résultats observés chez des sujets avec des altérations du système nerveux central.

Un premier groupe de recherches a été fait sur l'ainsi dite sensation spatiale acoustique déterminée par deux sons qui agissent sur les deux oreilles avec égale intensité (40 dB au dessus du seuil) et une très petite différence de fréquence (moins du 0,5 %). On obtient ainsi une période de modulation constante et inférieure à la minute seconde. Les fréquences employées ont été de 125 250 500 et 1000 Hz (Tableau 1). Des recherches préliminaires faites par Menzio et Schindler sur des jeunes auditivement normaux ont démontré qu'on peut réaliser des différentes sensations spatiales plus ou moins nettes selon les sujets et les bandes de fréquence employées. En

TABLEAU 2 *Types de sensation*

1		Sensation spatiale complète au tour de la tête
2		Sensation spatiale complète Au devant Au derrière Au dessus de la tête
3		Sensation spatiale incomplète (grosses flèches droites et gauches)
4		Sensation spatiale incomplète (légères droites et gauches)
5		Sensation spatiale incomplète Pas de localisation

## (ARTICULATION CURVE)

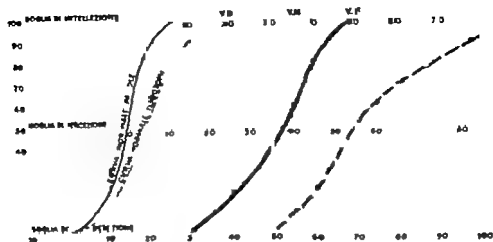


FIG. 2. Hypoxie voix accélérée — Normal ; courbe en hypoxie

silent des procès d'intégration neurale extrêmement délicats et précis. C'est la condition typique des sensations spatiales déterminée par deux sons avec petites différences de fréquence pour lesquelles en effet le jugement se base sur les seules différences de phase. Il faut rappeler à ce propos que par ce test on peut détecter des altérations précoces et ainsi de valeur diagnostique chez les syndromes de démyélinisation, les presbycusies et les traumatismes crâniens même légers. Des altérations, peut-être moins évidentes mais toujours significatives, ont été vues avec les tests de la voix avec distorsion et de la voix accélérée, qui se modifient surtout pour les lésions de l'aire acoustique et pour les lésions temporales profondes.

Des modifications inconstantes et modestes ont été relevées par le test dychoïque : pas d'altérations il y a eu par le test de la voix interrompue, qui indique généralement des lésions au niveau mésencéphalique. En conclusion il nous semble justifié croire que l'hypoxie peut déterminer des altérations fonctionnelles pour les procès qui se passent sur des circuits polysynaptiques surtout au niveau cortical ou sous-cortical.

## SUMMARY

In respiration for 5-10 min in mixture of 7.6% and 8.6% oxygen in  $N_2$ , the author has made the following tests: Acoustical space sensation by sounds of equal intensity and frequency difference dychoic test and vocal tests by distorted, accelerated, and interrupted voice. Significant alterations were noticed for space sensation and less for accelerated and interrupted voice. Oxygen diminution should determine functional modification for auditory processes which involve polysynaptic pathways and precise neural integration.

## (ARTICULATION CURVE)

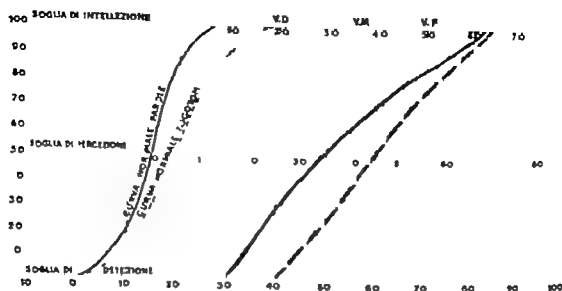


FIG. 1 Hypoxie - voix philtée — Normale; --- courbe en hypoxi

temps aux deux oreilles à une certaine intensité. Par l'examen des réponses on peut établir un pourcentage d'intelligibilité du test qui devrait donner des indications sur les capacités de discrimination et d'intégration acoustique à un niveau supérieur. La respiration des mélanges pauvres en  $O_2$  ne détermine que peu de modification aux réponses du test: la compréhension des messages verbaux est en effet toujours supérieure au 85% des mots transmis. Pour un seul cas, avec le mélange au 76% d' $O_2$  le pourcentage est descendu au dessous du 50%.

Une troisième série d'observations a été faite par les tests de la voix avec distorsion, voix accélérée et interrompue. Les sujets de cette série respiraient les mélanges pour 10 min.

Et voici les résultats:

**Voix avec distorsion.** Modification d'intelligibilité à basse intensité. En effet le seuil de détection et de perception étaient rejoints à une intensité de 15-20 dB supérieure aux épreuves effectuées avec respiration d'air normal tandis que le seuil d'intellection était rejoint à la même intensité qu'en conditions normales (Fig. 1).

**Voix accélérée.** Modifications de l'intelligibilité à toutes les intensités. On a jamais pu rejoindre le 100% d'intelligibilité avec la plus grande intensité de l'audiomètre: les courbes ont toujours une forme à plateau (Fig. 2).

**Voix interrompue.** Pas de modification.

Les observations que je viens de décrire ont une signification plutôt complexe et difficilement on peut en tirer des considérations de caractère conclusif. Cependant on peut affirmer que les altérations plus importantes déterminées par une chute de tension d' $O_2$  se réalisent pour les tests qui nécessitent

## [ARTICULATION CURVE]

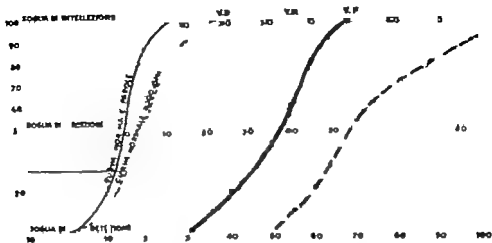


FIG. 2. Hypoxie — et accélération — N normale; -- courbe en hypoxie

sistent des procès d'intégration neurale extrêmement délicats et précis. C'est la condition typique des sensations spatiales déterminée par deux sons avec petites différences de fréquence pour lesquelles en effet le jugement se base sur les seules différences de phase. Il faut rappeler à ce propos que par ce test on peut déceler des altérations précoces et ainsi de valeur diagnostique chez les syndromes de démyélinisation, les presbycusies et les traumatismes crâniens même légers. Des altérations, peut être moins évidentes mais toujours significatives, ont été vues avec les tests de la voix avec distorsion et de la voix accélérée, qui se modifient surtout pour les lésions de l'aire acoustique et pour les lésions temporales profondes.

Des modifications inconstantes et modestes ont été relevées par le test dychotique pas d'altérations il y a eu par le test de la voix interrompue, qui indique généralement des lésions au niveau mésencéphalique. En conclusion il nous semble justifié croire que l'hypoxie peut déterminer des altérations fonctionnelles pour les procès qui se passent sur des circuits polysynaptiques surtout au niveau cortical ou souscortical.

## SUMMARY

In respiration for 3-10 min in a mixture of 7.5% and 8.5% oxygen in  $N_2$ , the author has made the following tests: Acoustical space sensation by sounds of equal intensity and frequency difference dychotic test and vocal tests by distorted, accelerated and interrupted voice. Significant alterations were noticed for space sensation and less for accelerated and interrupted voice. Oxygen diminution should determine functional modifications for auditory processes which involve polysynaptic pathways and precise neural integration.



## (ARTICULATION CURVE)

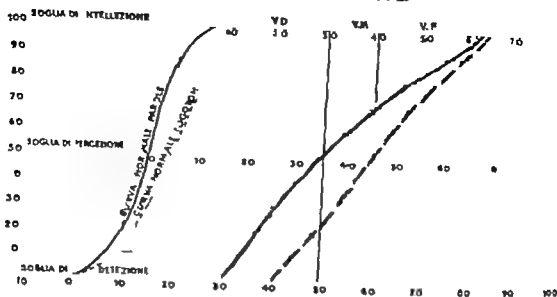


FIG 1 Hypoxie - voix philtree — Normale --- courbe en hypoxie

temps aux deux oreilles à une certaine intensité. Par l'examen des réponses on peut établir un pourcentage d'intelligibilité du test qui devrait donner des indications sur les capacités de discrimination et d'intégration acoustique à un niveau supérieur. La respiration des mélanges pauvres en  $O_2$  ne détermine que peu de modification aux réponses du test: la compréhension des messages verbaux est en effet toujours supérieure au 85% des mots transmis. Pour un seul cas, avec le mélange au 76% d' $O_2$ , le pourcentage est descendu au dessous du 50%.

Une troisième série d'observations a été faite par les tests de la voix avec distorsion, voix accélérée et interrompue. Les sujets de cette série respiraient les mélanges pour 10 min.

Et voici les résultats.

**Voix avec distorsion.** Modification d'intelligibilité à basse intensité. En effet le seuil de détection et de perception étaient rejoints à une intensité de 15-20 dB supérieure aux épreuves effectuées avec respiration d'air normal, tandis que le seuil d'intellection était rejoint à la même intensité qu'en conditions normales (Fig 1).

**Voix accélérée.** Modifications de l'intelligibilité à toutes les intensités. On a jamais pu rejoindre le 100% d'intelligibilité avec la plus grande intensité de l'audiomètre: les courbes ont toujours une forme à plateau (Fig 2).

**Voix interrompue.** Pas de modification.

Les observations que je viens de décrire ont une signification plutôt complexe et difficilement on peut en tirer des considérations de caractère conclusif. Cependant on peut affirmer que les altérations plus importantes déterminées par une chute de tension d' $O_2$  se réalisent pour les tests qui nécessitent

the most highly vascularized parts of the whole central nervous system. In my own investigation of children dying from asphyxia I found a severe cell loss in these nuclei, up to more than 60% of the cells were lost in the dorsal nucleus. Changes of this order were not found anywhere else in the auditory system.

*P. Menlo (Réponse) à Mr Holmgren* Il est probable que les altérations, dans la compréhension d'un message verbal, caractéristiques des patients presbiacousiques soumis à hypoxie soient dues au fait que les modifications anatomo-pathologiques et fonctionnelles du vieillard sont localisées soit à niveau de l'organe de Corti, soit à niveau des voies auditives centrales. Dans ces conditions l'hypoxie peut déterminer des altérations remarquables dans les processus d'intégration neuronale.

Un appui à cette interprétation est représenté par le fait que l'hypoxie ne détermine pas des modifications du seuil pour les tons purs, mais dans la compréhension des mots, des logatomes et des phrases.

En ce qui concerne les erreurs dans la dactylographie, déterminées par l'hypoxie, je crois que la chaîne des phénomènes (idéatif et d'élaboration motrice) qui sont indispensables pour qui pratique la dactylographie et qui nécessitent l'action de circuits polysynaptiques très compliqués, soient facilement troublés par une modification de la tension partielle d'O<sub>2</sub> et par la conséquente altération métabolique.

Et enfin, pour ce que concerne la troisième question, je pense que les modifications observées à l'audiométrie vocale soient secondaires à variations fonctionnelles soit à niveau cochléaire que sous-cortical.

À Mr Hall Je suis d'accord avec lui sur l'importance des noyaux cochléaires, surtout du noyau accessoire qui a fait l'objet d'études particulièrement importantes, surtout au point de vue électrophysiologique et anatomopathologique.

J pense toutefois que les résultats obtenus par les différentes techniques, et par ceux que j'ai référés en précédence, font supposer que l'hypoxie détermine des modifications fonctionnelles à des niveaux différents, mais surtout à des niveaux corticaux et sous-corticaux. Seulement des recherches systématiques, électrophysiologiques, linéiques, expérimentelles et pharmacologiques (substances neurotropes) pourront nous donner des renseignements précis sur l'importance des différentes structures.

## ZUSAMMENFASSUNG

Durch Atmung einer Mischung während 5-10 Minuten von 7,5 bzw 8,6 O<sub>2</sub> in Stickstoff hat der Verfasser folgende Tests untersucht akustische Raumsensation durch Töne gleicher Intensität aber ungleicher Frequenz dychothischer Test vokale Tests mit Distorsion akzellerierter und unterbrochener Stimme Bedeutende Änderungen wurden für die Raumsensation verursacht weniger für die akzellierte und unterbrochene Stimme Die Sauerstoffverminderung verursacht funktionelle Veränderungen besonders für die Prozesse welche polysynaptische Wege und scharfe neurale Integration gebrauchen

## BIBLIOGRAPHIE

- CHANCE, H CORNEX P JOHNS, F et SCHÖNBER, H 1962: Intracellular oxidation reduction state *in vivo* *Science* 137 490
- DAVIES, P W et GREENELL, R G 1962: Metabolism and function in the cerebral cortex under local perfusion with the aid of an oxygen cathode for surface measurement of cortical oxygen consumption *J Neurophys* 25 661
- LETT S S 1953 Cerebral circulation Symposium on circulation and homeostasis *Medical Sciences Publication* 3 180
- MIRABAY G A HILDRETH J M SHIVABANGER, E W CLARK, L C, et RICE, E A, 1958: Endolymphatic oxygen tension in the cochlea of the guinea pig *Ibid.* 247
- MIRABAY G A DE JONGE, B R, SHIVABANGER, E W et ARNOLD, J E, 1958 Effect of localized hypoxia in the electrophysiological activity of the cochlea of the guinea pig *J Acoust Soc. Am* 705
- MIRABAY G A SHIVABANGER, E W et ARNOLD, J E, 1958: Changes in cochlea endolymphatic oxygen availability action potential and microphonics during a different physiological hypoxia and exposure to sound *Ibid* 30 701
- REIVICH, M 1964: Arterial PCO<sub>2</sub> and cerebral hemodynamics *Amer J Physiol* 206 35
- TAUOG, M et PERLMAN H B, 1965: Cochlea oxygen tension Relation to blood flow and function *Acta Otolaryng* (Stockh.) 59 487

Piazza Cavour 19  
10123 Torino 11 ly

## DISCUSSION

L. Holmgren (1) Could this explain the well-known fact that individuals with presbycusis have increased hearing difficulties in an atmosphere which gives hypoxia, as exists in the presence of intense smoking, and consequently an overload of carbon oxide? (2) Has the author made correlations with other semi psychological tests such as typewriting, where the percentage of mistakes increases under the influence of hypoxia? (3) Are the changes of the speech-audiogram entirely due to the influence of hypoxia in the subcortical region or also in the inner ear?

J G Hall Mr Menzio said that the results of asphyxia may be cortical or subcortical. We have some evidence that the greatest damage is in the cochlear nuclei. Croigie has counted the number of capillaries in the auditory system, and found the cochlear nuclei, especially the dorsal cochlear nucleus, to be among



FIG. 1 Cochleogram from guinea pig sacrificed 1 week following irradiation. Open circles represent apparently normal cells, solid black circles represent degenerated cells. Left side irradiated with 8000 R.

Dose rate at the site of the inner ear was 200 R/min. The X-ray dose was delivered to the left half of the skull through a rectangular opening (20 mm  $\times$  17 mm) in a leaden sheet (10.5 mm) which also shielded the rest of the animal. The right inner ear served as control. During irradiation the animal was placed in a cage which did not permit movements of the head. Narcosis was not given. The doses applied were 1000 R, 2000 R, 4000 R, 6000 R, and 7000 R. The animals were sacrificed 3 hours, 6 hours, 18 hours and 1 week after the irradiation. Investigations were performed by phase contrast microscopy, ordinary light microscopy and electron microscopy.

The inner ear was fixed in chilled, coronal buffered 1.5% osmium tetroxide solution and kept refrigerated at 40 C for 1-2 hours. For examination by phase contrast microscopy samples of each of the four coils of the organ of Corti (Engström *et al.* 1966) and the sensory areas of the cristae and the maculae (Lindeman, 1967) were dissected and mounted in glycerine. By this method a bird's-eye view of a large number of sensory and supporting cells may be obtained. By focussing the field of vision, an optical sectioning of the specimen may be performed, permitting a study of different parts of the sensory and supporting cells. Ordinary light microscopy was performed on stained sections of paraffin- or araldite-embedded material. For the electron microscopy the cristae and the maculae and parts from each coil of the organ of Corti were dissected and embedded in epon or araldite, sectioned on an LKB ultramicrotome and mounted on copper grids with formvar coating. The sections were then stained with uranyl acetate and lead citrate (Reynolds, 1963).

# EARLY DEGENERATIVE CHANGES IN THE INNER EAR SENSORY CELLS OF THE GUINEA PIG FOLLOWING LOCAL X RAY IRRADIATION

## 1 Preliminary Report

FINN Ø WINTHER

*From the Department of Oto-rhino-laryngology (Rikshospitalet) and Anatomical Institute University of Oslo Oslo Norway*

X ray irradiation in single doses was applied to the inner ear of guinea pigs. The organ of Corti and the vestibular end organs were examined by light microscopy using the surface specimen technique proposed by Engström. A characteristic pattern of degeneration was found. The outer hair cells of the two basal coils of the cochlea were extensively degenerated whereas the outer hair cells of the two apical coils and the inner hair cells were intact. In the vestibular end organ sensory cells of the periphery of the cristae ampullares and the maculae utriculi and sacculi proved to be more vulnerable than those of the central parts. A preliminary report of an electronmicroscopic study of the early degenerative changes is given.

The effect of ionizing radiation on the inner ear has previously been studied by histological and functional methods (for references see Kelemen, 1963). The histological examination of the irradiated inner ears has, however, been performed on stained sections with ordinary light microscopy. The results of these studies have been controversial and a re-investigation of the problem with new methods was therefore thought to be of interest. In the present investigation, the surface specimen technique recently applied by Engström (Engström *et al.*, 1966; Lindeman, 1967) was adapted and in addition light and electron microscopy on stained sections was used in order to get an answer to the following questions:

- 1 Does irradiation of the inner ear with X rays cause any damage to the sensory cells?
- 2 Do X rays bring about any characteristic pattern of damage?
- 3 Which are the first signs of damage and how soon after the irradiation are these seen?

## MATERIAL AND METHODS

Guinea pigs were used as experimental animals. The source of radiation was a Siemens Stabilipan roentgen apparatus operated at 200 kV, 12 mA, and equipped with a Thoraeus filter II (0.8 mm Sn, 0.25 mm Cu, 1 mm Al).

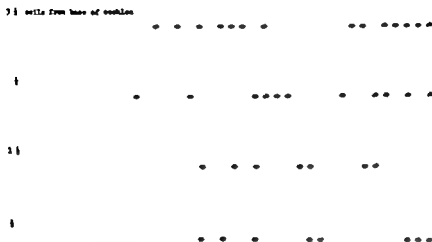


FIG. 1 Cochleogram from guinea pig sacrificed 1 week following irradiation. Open circles represent apparently normal cells, solid black circles represent degenerated cells. Left side irradiated with 8000 R.

Dose rate at the site of the inner ear was 200 R/min. The X-ray dose was delivered to the left half of the skull through a rectangular opening (20 mm  $\times$  17 mm) in a leaden sheet (10.5 mm) which also shielded the rest of the animal. The right inner ear served as control. During irradiation the animal was placed in a cage which did not permit movements of the head. Narcosis was not given. The doses applied were 1000 R, 2000 R, 4000 R, 6000 R, and 7000 R. The animals were sacrificed 3 hours, 6 hours, 18 hours and 1 week after the irradiation. Investigations were performed by phase contrast microscopy, ordinary light microscopy and electron microscopy.

The inner ear was fixed in chilled veronal buffered 1.5% osmium tetroxide solution and kept refrigerated at 40 C for 1-2 hours. For examination by phase contrast microscopy samples of each of the four coils of the organ of Corti (Engström *et al.*, 1968) and the sensory areas of the cristae and the maculae (Lindeman, 1967) were dissected and mounted in glycerine. By this method a bird's-eye view of a large number of sensory and supporting cells may be obtained. By focussing the field of vision, an optical sectioning of the specimen may be performed, permitting a study of different parts of the sensory and supporting cells. Ordinary light microscopy was performed on thin sections of paraffin or araldite-embedded material. For the electron microscopy the cristae and the maculae and parts from each coil of the organ of Corti were dissected and embedded in epon or araldite, sectioned on an LKB ultramicrotome and mounted on copper grids with formvar coating. The sections were then stained with uranyl acetate and lead citrate (Reynolds, 1963).



FIG. 2. Surface specimen from the organ of Corti of the guinea pig, 1 coil from the base of the cochlea. The animal was irradiated with 6000 R and sacrificed 1 week later. Phase contrast micrograph. Focal plane at the level of the cuticular plates. IHC, inner hair cells, 1, 2, and 3. First, second and third row of outer hair cells. Note that the inner hair cells are intact, and that most of the outer hair cells are degenerated, showing the typical "collapse" figure (arrow).  $\times 700$ , obj 100, oc 8.

FIG. 3. Surface specimen from the periphery of the crista ampullaris lateral of guinea pig irradiated with 7000 R and sacrificed 1 week following irradiation. Phase contrast micrograph. Focal plane at the level of the cuticular plates. The degenerated sensory cells show the typical "collapse" figure (arrow).  $\times 700$ , obj 100, oc 8.

## RESULTS

The first question (does irradiation of the inner ear with X rays cause any damage to the sensory cells?) could be clearly answered with yes. When doses of 6000 R and 7000 R were applied degenerated sensory cells in a number exceeding that on the not irradiated side was evident. Also the second question (do X rays bring about any characteristic pattern of damage?) could be answered with yes. Phase contrast microscopy of the organ of Corti showed that extensive degeneration was found in the outer hair cells of the basal coil and usually also in the outer hair cells of the second coil from the base of the cochleae (Figs 1 and 2). The outer hair cells of the two apical coils and the inner hair cells were normal. In the vestibular sensory epithelium the periphery of the cristae and the maculae showed the greatest number of degenerated cells while the number of degenerated cells in the central areas were clearly smaller (Fig 3). No clear difference in the number of degenerated sensory cells between the cristae and the maculae was found although there was a tendency toward a smaller number of degenerated sensory cells in the maculae sacculi than in the maculae utriculi and the cristae ampullares. The question whether the degenerated vestibular sensory cells were of the type I or II could not be revealed with certainty by light microscopy. By electron microscopy however degenerated sensory cells both of type I and II were found.

By phase contrast microscopy of animals irradiated with 7000 R and sacri

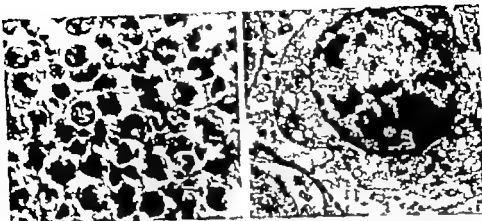


FIG. 4. A face specimen from the periphery of the crista ampullaris lateralis of guinea pig irradiated with 7000 R and sacrificed 3 hours following irradiation. Phase contrast micrograph. Focal plane at the level of the sensory cell nuclei. Arrows point to nuclei with coarse granulation. 800, obj 100, oc. 10.

FIG. 5. Sensory cell type II from the crista ampullaris lateralis of guinea pig irradiated with 7000 R and sacrificed 3 hours following irradiation. Clumping of the bromatol of the nucleus is seen and numerous granules of moderate size are present in the cytoplasm. 750x.

sified 3 hours following the irradiation an answer was given to the third question (what are the first signs of damage and how soon after the irradiation are these seen?) The nuclei of the outer hair cells of the basal coil of the organ of Corti were coarsely granulated but had retained their shape and size. In animals sacrificed 6 hours following irradiation many of the nuclei of the basal coils were pyknotic. In these cells changes which ranged from a slight distortion of the normal W-pattern of the sensory hairs to incipient formation of a spiderlike degeneration figure in the cuticular region was found. Eighteen hours following irradiation completely degenerated outer hair cells were found in the two basal coils.

Phase contrast microscopy of the vestibular epithelium of animals irradiated with 7000 R showed that 3 hours following the irradiation some of the sensory cells of the periphery had a coarsely granulated nucleus (Fig. 4). In these cells the sensory hair bundles were usually distorted and often a spiderlike degeneration figure in the cuticular plane was seen. At 6 hours following irradiation all stages of degeneration from a slight coarse granulation of the nucleus to completely degenerated sensory cells were observed. Eighteen hours following irradiation many totally degenerated sensory cells were found in the periphery of the cristae and the maculae.

The early degenerative changes were also studied by electron microscopy. Only a small number of animals have so far been examined, and the results presented are therefore preliminary.

In its broad features the mode of degeneration seemed to be the same in the sensory cells of the organ of Corti and the sensory cells of the vestibular





FIG. 2 Surface specimen from the organ of Corti of the guinea pig, 1 / coils from the base of the cochlea. The animal was irradiated with 6000 R and sacrificed 1 week later. Phase contrast micrograph. Focal plane at the level of the cuticular plates. IUC, inner hair cells, 1 2, and 3 First, second and third row of outer hair cells. Note that the inner hair cells are intact and that most of the outer hair cells are degenerated, showing the typical "collapse" figure (arrow)  $\times 700$ , bj 100, oc 8.

FIG. 3 Surface specimen from the periphery of the crista ampullaris lateralis of a guinea pig irradiated with 7000 R and sacrificed 1 week following irradiation. Phase contrast micrograph. Focal plane at the level of the cuticular plates. The degenerated sensory cells show the typical "collapse" figure (arrow)  $\times 700$ , bj 100, oc 8.

## RESULTS

The first question (does irradiation of the inner ear with X rays cause any damage to the sensory cells?) could be clearly answered with yes. When doses of 6000 R and 7000 R were applied degenerated sensory cells in a number exceeding that on the not irradiated side was evident. Also the second question (do X rays bring about any characteristic pattern of damage?) could be answered with yes. Phase contrast microscopy of the organ of Corti showed that extensive degeneration was found in the outer hair cells of the basal coil and usually also in the outer hair cells of the second coil from the base of the cochleae (Figs 1 and 2). The outer hair cells of the two apical coils and the inner hair cells were normal. In the vestibular sensory epithelium the periphery of the cristae and the maculae showed the greatest number of degenerated cells while the number of degenerated cells in the central areas were clearly smaller (Fig 3). No clear difference in the number of degenerated sensory cells between the cristae and the maculae was found although there was a tendency toward a smaller number of degenerated sensory cells in the maculae sacculi than in the maculae utriculi and the cristae ampullares. The question whether the degenerated vestibular sensory cells were of the type I or II could not be revealed with certainty by light microscopy. By electron microscopy however degenerated sensory cells both of type I and II were found.

By phase contrast microscopy of animals irradiated with 7000 R and sacri

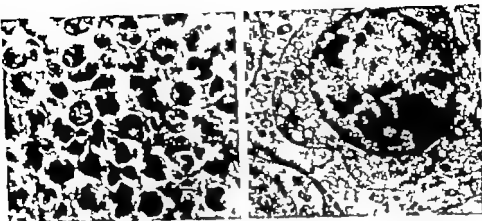


FIG. 4. Surface specimen from the periphery of the cristae ampullari lateralis of guinea pig irradiated with 7000 R and sacrificed 3 hours following irradiation. Phase contrast micrograph. Focal plane at the level of the sensory cell nuclei. Arrows point to nuclei with coarse granulation. 300, obj 100, oc. 10.

FIG. 5. Sensory cell type II from the cristae ampullari lateralis of guinea pig irradiated with 7000 R and sacrificed 3 hours following irradiation. Clumping of the chromatin of the nucleus is seen and numerous vacuoles of moderate size are present in the cytoplasm. 7,500.

3 hours following the irradiation an answer was given to the third question (what are the first signs of damage and how soon after the irradiation are these seen?) The nuclei of the outer hair cells of the basal coil of the organ of Corti were coarsely granulated but had retained their shape and size. In animals sacrificed 6 hours following irradiation many of the nuclei of the basal coils were pycnotic. In these cells changes which ranged from a slight distortion of the normal V pattern of the sensory hairs to incipient formation of a spider-like degeneration figure in the cuticular region was found. Eighteen hours following irradiation completely degenerated outer hair cells were found in the two basal coils.

Phase contrast microscopy of the vestibular epithellum of animals irradiated with 7000 R showed that 3 hours following the irradiation some of the sensory cells of the periphery had a coarsely granulated nucleus (Fig. 4). In these cells the sensory hair bundles were usually distorted and often a spiderlike degeneration figure in the cuticular plane was seen. At 6 hours following irradiation all stages of degeneration from a slight coarse granulation of the nucleus to completely degenerated sensory cells were observed. Eighteen hours following irradiation many totally degenerated sensory cells were found in the periphery of the cristae and the maculae.

The early degenerative changes were also studied by electron microscopy. Only a small number of animals have so far been examined, and the results presented are therefore preliminary.

In its broad features the mode of degeneration seemed to be the same in the sensory cell of the organ of Corti and the sensory cells of the vestibular



FIG. 2. Surface specimen from the organ of Corti of the guinea pig. 1/ colls from the base of the cochlea. The animal was irradiated with 6000 R and sacrificed 1 week later. Phase contrast micrograph. Focal plane at the level of the cuticular plates. IHC, inner hair cells, 1, 2, and 3, First, second and third row of outer hair cells. Note that the inner hair cells are intact, and that most of the outer hair cells are degenerated, showing the typical "collapse" figure (arrow)  $\times 700$ , obj 100, oc 8.

FIG. 3. Surface specimen from the periphery of the crista ampullaris of the guinea pig irradiated with 7000 R and sacrificed 1 week following irradiation. Phase contrast micrograph. Focal plane at the level of the cuticular plates. The degenerated sensory cells show the typical "collapse" figure (arrow)  $\times 700$ , obj 100, oc 8.

## RESULTS

The first question (does irradiation of the inner ear with X rays cause any damage to the sensory cells?) could be clearly answered with yes. When doses of 6000 R and 7000 R were applied degenerated sensory cells in a number exceeding that on the not irradiated side was evident. Also the second question (do X rays bring about any characteristic pattern of damage?) could be answered with yes. Phase contrast microscopy of the organ of Corti showed that extensive degeneration was found in the outer hair cells of the basal coil and usually also in the outer hair cells of the second coil from the base of the cochlea (Figs. 1 and 2). The outer hair cells of the two apical coils and the inner hair cells were normal. In the vestibular sensory epithelium the periphery of the cristae and the maculae showed the greatest number of degenerated cells while the number of degenerated cells in the central areas were clearly smaller (Fig. 3). No clear difference in the number of degenerated sensory cells between the cristae and the maculae was found although there was a tendency toward a smaller number of degenerated sensory cells in the maculae sacculi than in the maculae utriculi and the cristae ampullares. The question whether the degenerated vestibular sensory cells were of the type I or II could not be revealed with certainty by light microscopy. By electron microscopy however degenerated sensory cells both of type I and II were found.

By phase contrast microscopy of animals irradiated with 7000 R and sacri

A characteristic pattern of sensory cell degeneration was found both in the organ of Corti and the vestibular epithelium.

In the organ of Corti the outer hair cells of the basal coils were found to be most vulnerable to X-ray irradiation. It is interesting to note that the same cell group also was affected first when guinea pigs were given large doses of antibiotics of the streptomycetes group (Hawkins & Engström, 1963; Kohonen, 1965). In the vestibular sensory epithelium however the greatest sensitivity to X-ray irradiation was in the periphery of the cristae and the maculae. Here however the largest damage caused by antibiotics of the streptomycetes group is found in the central regions (Lindeman, 1967). These variations in vulnerability of various cells are not readily explicable but may possibly be related to differences in morphology and biochemistry.

The first effect of radiation on living matter is physical in that it affects atoms and molecules irrespective of their arrangement in living structures, resulting in splitting of the molecules into radicals and ions. The chemical balance of the cells is thus upset. A possible explanation of the sequence of events observed by electron microscopy is that the DNA/RNA system is affected resulting in vacuolization of the endoplasmatic reticulum due to an abnormal protein synthesis.

Referring to this it is interesting that instillation into the guinea pig bulla tympanicum of streptomycin, which interferes with the protein synthesis (Gorini, 1966) gives a similar electron microscopic picture (Spoendlin, 1966) as that observed following X-ray irradiation.

# RESUME

L'irradiation par des rayons X fut appliquée à dose faiblée de a l'oreille interne du cobay. L'organe de Corti et l'extrémité des organes vestibulaires furent examinés de : l'éclairag microscop que en utilisant la tech lique des spécimens de surface : proposés pa Engström. Un genre de dégénéral a caractéristique fut trou é. Les cellules ciliées externes des deux tours de spin : de la cochlée situées à la base étaient dégénérées d façon pron ootée, tandis que les cellules ciliées externes appartenant a deux tours apicaux et les cellules ciliées internes étaient intactes. Dans l'organe terminal vestibulaire, les cellules sensori les de la périphéri du cristae amp llares, du maculae utriculi et du saccull, démontrent qu elles sont plus vulnérables que celles de la partie centrale. Un reportage préliminaire aussi été f il, concerne l'étude au microscope électronique des altérations dégénératives précoces.

# ZUSAMMENFASSUNG

Die Wirkung einer Einzeldosis von Röntgenstrahlen uf di Sinnes-Zellen des inneren Ohres von Meerschwein chen wurde lichtmikroskopisch an Präparaten untersucht, di sich der Methode von Engström hergestellt waren. E konnte el charakteristisches Degenerationmuster festgest ill werden. Di inneren Haarzellen der beiden basalen Schneckenwindungen zeigten ausgedeh lte degene-

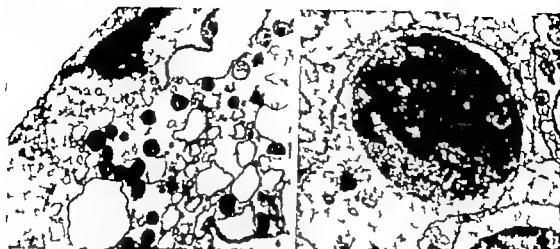


FIG. 6 Subcuticular region of an outer hair cell of the organ of Corti / a cell from the base of a guinea pig cochlea. The animal was irradiated with 7000 R and sacrificed 3 hours later. Marked vacuolisation of the cytoplasm. The cuticular plate is intact.  $\times 12,500$

FIG. 7 Sensory cell type II from the crista ampullaris lat. rali of a guinea pig irradiated with 7000 R and sacrificed 6 hours later. The nucleus is deformed and the chromatin clotted in dense clumps. The cytoplasm is highly electron-dense. A few vacuoles (arrows) and ribosome-like granules are visible in the cytoplasm.  $\times 10,000$

epithelium. As was assumed from light microscopy, the first sign of damage to the cell was a clumping of the chromatin granules of the nucleus. In many cells this was found without any changes of the cytoplasmic organelles. In other cells where the nuclear changes were more pronounced, there were cytoplasmic vacuoles, often of considerable size and with the surface lined with ribosomelike granules (Figs. 5 and 6). At this stage swollen mitochondria, often with disrupted cristae, vacuole formation, and myelin figures could be found side by side with apparently normal mitochondria. A special feature of the vestibular sensory cells was expulsion of portions of the cytoplasm on the endolymphatic surface. Before the ultimate resorption of the degenerated sensory cell the cytoplasm and nucleus underwent pyknosis (Fig. 7). Except for a few vacuoles and ribosome-like granules, details could not be recognized in the highly electron-dense cell. This stage of degeneration was reached within 6 hours from the completion of irradiation.

#### DISCUSSION

Cell degeneration following exposure to X rays may be caused by a direct action in the degenerating cell, or it may be caused by interference with the blood supply. A third possibility is a combination of these factors. During the preparation of the temporal bones no gross damage to the vessels resulting in hemorrhage or exudate was noted. Neither did preliminary light and electron microscopic study of the vessels reveal any damage. The functional state of the vessels however cannot be judged from histological specimens alone (Kreyberg 1928).

A characteristic pattern of sensory cell degeneration was found both in the organ of Corti and the vestibular epithelium

In the organ of Corti the outer hair cells of the basal coils were found to be most vulnerable to X-ray irradiation. It is interesting to note that the same cell group also was affected first when guinea pigs were given large doses of antibiotics of the streptomycetes group (Hawkins & Engström, 1963; Kohonen, 1965). In the vestibular sensory epithelium however the greatest sensitivity to X-ray irradiation was in the periphery of the cristae and the maculae. Here however the largest damage caused by antibiotics of the streptomycetes group is found in the central regions (Lindeman, 1967). These variations in vulnerability of various cells are not readily explicable but may possibly be related to differences in morphology and biochemistry.

The first effect of radiation on living matter is physical in that it affects atoms and molecules irrespective of their arrangement in living structures, resulting in splitting of the molecules into radicals and ions. The chemical balance of the cells is thus upset. A possible explanation of the sequence of events observed by electron microscopy is that the DNA/RNA system is affected resulting in vacuolisation of the endoplasmatic reticulum due to an abnormal protein synthesis.

Referring to this it is interesting that instillation into the guinea pig bulla tympanicum of streptomycin, which interferes with the protein synthesis (Gorini, 1966) gives a similar electron microscopic picture (Spoendlin, 1966) as that observed following X-ray irradiation.

# RESUME

L'irradiation par des rayons X fut appliquée à dose isolée dans l'oreille interne du cobaye. L'organe de Corti et l'extrémité des organes vestibulaires furent examinés dans l'éclairage microscopique en utilisant la technique des spécimens de surface proposée par Engström. Un genre de dégénération caractéristique fut trouvé. Les cellules ciliées externes des deux tours de spire de la cochlée atteintes à la base étaient dégénérées de façon prononcée, tandis que les cellules ciliées externes appartenaient à un deuxième tour spiral et les cellules ciliées internes étaient intactes. Dans l'organe terminal vestibulaire, les cellules sensorielles de la périphérie du crista ampullaire, du macula utriculi et du sacculi, démontrent qu'elles sont plus vulnérables que celles de la partie centrale. Un reportage préliminaire aura été fait, concernant l'étude au microscope électronique des altérations dégénératives précoces.

# ZUSAMMENFASSUNG

Die Wirkung einer Einzeldosis von Röntgenstrahlen auf die Sinneszellen des inneren Ohres vom Meerschweinchen wurde histologisch an Präparaten versucht. Nach der Methode von Engström hergestellt waren. Es konnte ein charakteristisches Degenerationsmuster festgestellt werden. Die äusseren Haarzellen der beiden basalen Schneckendrehungen zeigten ausgesprochenen

relative Veränderungen während die entsprechenden Zellen der beiden apikalen Windungen und die inneren Haarzellen normal waren. In dem vestibulären Teil des inneren Ohres zeigten die Sinnes-Zellen in der Peripherie der Cristae ampullares und der Maculae utriculi und sacculi grössere Vulnerabilität als die entsprechenden Zellen in den zentralen Regionen. Ein Präliminar Rapport über das Elektronenmikroskop-Studium der frühen degenerativen Veränderungen ist gegeben.

## REFERENCES

- ENGSTRÖM H, ADER, H and ANDERSSON A 1966: *Structural pattern of the organ of Corti* Almqvist & Wiksell Stockh. Im.  
 GONIEL, L. 1966: Antibiotics and the genetic code *Sci Amer* 214 102.  
 HAWKINS, J. E., JR and ENGSTRÖM, H 1963 Effect of kanamycin on cochlear electroarchitecture *Acta Otolaryng* (Stockh.) Suppl. 188  
 KELEMAN, G 1963 Radiation and ear *Acta Otolaryng* (Stockh.) Suppl. 184  
 KOHONEN A 1963: Effect of some ototoxic drugs upon the pattern and innervation of the cochlear sensory cells in the guinea pig *Acta Otolaryng* (Stockh.) Suppl. 203  
 KREYBERG, L., 1928: Über präcanceröse Gefässveränderungen *Vierteljahrsschrift für gerichtliche und allgemeine Pathologie* 273 367  
 LINDEMAN, H. H. 1967 Cellular pattern and nerve supply of the vestibular sensory epithelia *Acta Otolaryng* (Stockh.) Suppl. 224  
 REYNOLDS, E. S 1963: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy *J Cell Biol* 17 208  
 SPÖRDLIN H 1966 Some morphological and pathological aspects of the vestibular sensory epithelia *Second symposium of the role of the vestibular organs in space exploration* National Aeronautics and Space Administration, SP 115 89

Dept. of Otolaryngology  
 Rikshospitalet Ost. Örebro

## OTOSCLEROSIS AND OSTEOGENESIS IMPERFECTA

P. BRETLAU and M. BALSLEV-JØRGENSEN

*From the Department of Otolaryngology Rigshospitalet (The University Hospital)  
Copenhagen Denmark*

The possibility of a common aetiology of otosclerosis and osteogenesis imperfecta is discussed. The histopathological differences in two temporal bones, one with osteogenesis imperfecta congenita and one with co-existing osteogenesis imperfecta tarda and otosclerosis, are emphasized. On the basis of the method currently used it cannot be confirmed, that otosclerosis and osteogenesis imperfecta have a common aetiology.

In the effort to find the early changes in otosclerosis and to view otosclerosis as a manifestation of generalized disease attention has been focussed, since the beginning of this century on well-known bony dystrophies, such as osteitis fibrosa or Recklinghausen's disease, osteitis deformans or Paget's diseases, and osteogenesis imperfecta.

Clinically and aetiologicaly these diseases differ but histologically they are similar in many respects.

In cases of incipient otosclerosis histological examination reveals osteoclastic destruction of the bony tissue in the labyrinthine capsule followed by new formation of abnormal bony tissue. The latter presents itself as tissue of a mosaic pattern with large medullary cavities and cellular highly vascularized bony tissue poor in fibrils. However a finding of destruction and new formation of bony tissue at the same time is typical of all vital bony changes.

A possible relationship between otosclerosis and osteogenesis imperfecta has been a subject of quite particular interest.

Osteogenesis imperfecta is a relatively uncommon, inherited, generalized connective-tissue disease. The characteristic syndrome of blue sclerae brittle bones with a tendency to fractures, and hearing loss of a conductive type is generally related to the names of van der Horst and de Kleijn who were the first to suggest, in 1918, that the hearing loss was due to otosclerosis. Rutlin (1922), Nagel (1922) and Gimplinger (1926) are said to have been the first to claim that they could confirm this histologically.

The heredity is believed to be by a dominant Mendelian trait (Seedorf 1919).

Weber in 1920, found in the callus in a healing fracture in a patient with osteogenesis imperfecta, bony changes highly reminiscent of the so-called blue mantles in typical otosclerotic foci. When considering also the mode of



rative Veränderungen während die entsprechenden Zellen der beiden apikalen Windungen und die inneren Haarzellen normal waren. In dem vestibulären Teil des inneren Ohres zeigten die Sinnes-Zellen in der Peripherie der Cristae ampullares und der Maculae utriculi und sacculi grössere Vulnerabilität als die entsprechenden Zellen in den zentralen Regionen. Ein Präliminar Rapport über das Elektronenmikroskop-Studium der frühen degenerativen Veränderungen ist gegeben.

## REFERENCES

- EXOSTRÖM H, ADEA, H and ANDERSSON, A 1966: *Structural pattern of the organ of Corti*. Almqvist & Wiksell Stockholm.
- GORINI, L. 1966 Antibiotics and the genetic code *Sci Amer* 214 102.
- HAWKINS, J E., JR., and EXOSTRÖM H 1963: Effect of kanamycin on cochlear microstructure *Acta Otolaryng* (Stockh.) Suppl 188
- HELEMAN G 1963: Radiation and ear *Acta Otolaryng* (Stockh.) Suppl 184
- KOHONEN A 1965 Effect of some ototoxic drugs upon the pattern and innervation of the cochlear sensory cells in the guinea pig *Acta Otolaryng* (Stockh.) Suppl 204
- KREYENBURG, L., 1928 Über präkaneröse HÖrveränderungen *Virchows Arch Path. Anat* 273 367
- LINDEMAN, H H 1967: Cellular pattern and nerve supply of the vestibular sensory epithelia. *Acta Otolaryng* (Stockh.) Suppl 224
- REYNOLDS, E. S 1963 The use of lead citrate at high pH as an electron-opaque stain in electron microscopy *J Cell Biol* 17 208
- SPOENDLIN, H 1966 Some morphological and pathological aspects of the vestibular sensory epithelia *Second symposium of the role of the vestibular organs in space exploration* National Aeronautics and Space Administration, SP 114, 99

Dept of Otorhinolaryngology  
Rikshospital i Oslo Norway



FIG. 3. Fissula ante fenestram, tectorial ligament, and stapes  $\times$  otosclerosis or fixation of the stapes (case 1)

FIG. 4. Inner layer of the labyrinthine capsule round the superior semicircular canal. A narrow endosteal layer and a thickened endochondral layer  $\times$  marrow medullary canals and cartilage remnant in the immature bony tissue (case 1)

centrally around the cochlear lumen and a somewhat thicker more continuous layer peripherally. Between these two layers there were bone marrow spaces. The ground substance consisted of few irregularly arranged fibres. Osteocytes were of irregular shape and arrangement. The amount of cartilaginous tissue was relatively increased (Fig. 2).

The periosteal capsule was poorly developed, consisting of a cellular web-like bony tissue of a somewhat coarser fibrillar structure than the endochondral layer. In several places the cells were large with vesicular nuclei.

However there was a gradual transition between the peripheral part of the endochondral layer and the periosteal layer, the latter showing a denser fibrillar structure.

The vestibular part of the labyrinthine capsule showed approximately the same appearance. The endosteal layer was poorly developed and the endochondral layer here too, divided into a less continuous layer centrally and a thicker continuous layer peripherally—with large cartilaginous islets, especially around the fissula ante fenestram (Fig. 3). In this part too the periosteal layer was thin.

Similar changes were found in the capsule surrounding the semicircular canal (Fig. 4) with a faint, but normal developed endosteal layer. The endochondral layer was of the same structure as in other areas of the labyrinthine capsule.

#### Case 2

Both temporal bones from a 77-year-old man. Clinical diagnosis: Osteogenesis imperfecta tarda.

In the labyrinthine capsule on both sides severe abnormalities, and two types of abnormal tissue could be distinguished (Fig. 5).

Primarily pronounced otosclerotic changes, about threequarters of the



FIG. 1 Labyrinthine capsule with congenital osteogenesis imperfecta. Cochlea, part of the vestibule and the footplate of the stapes (case 1)



FIG. 2 Basal turn of cochlea. Between the triangular scularis and the immature bone matrix a well marked layer of chondrocytes (case 1)

inheritance and the common clinical co-existence of the two diseases, this finding pointed to a common genesis.

In recent years Simson Hall and his associates (1961, 1962) as well as Wullstein (1960) have studied the relationship between osteogenesis imperfecta and otosclerosis. They arrived at the conclusion that the two diseases are due to a common genetic anomaly in the osteoblasts, otosclerosis being interpretable as a localized form of osteogenesis imperfecta, differing from it only in degree, extent and localization.

In contradistinction Altmann *et al.* (1962, 1967) in particular have not in their own studies or in the literature on the subject been able to find changes suggesting otosclerosis in temporal bones affected with osteogenesis imperfecta. Accordingly they do not feel there is histological or histochemical evidence of a common aetiology, but the frequent co-existence of the two diseases might perhaps be due to osteogenesis imperfecta predisposing to the development of otosclerosis by way of a still unknown chemical or ultramicroscopic structural abnormality.

In a study of the temporal bones in our laboratory we have found two cases of osteogenesis imperfecta.

Since one of these cases also exhibited otosclerotic changes, we felt that our findings were worthy of publication.

### *Report of Cases*

#### *Case 1*

Both temporal bones from a newborn infant with osteogenesis imperfecta.

In the bony cochlear capsule (Fig. 1) the endosteal layer was very thin but normal. A continuous, darker line separated this layer from the endochondral layer which was well-developed and made up of a delicate skein-like, immature, and irregular bony tissue with ample newformation of bone. The endochondral layer formed a thin, more or less continuous layer



Fig. 2. *Left fenestra, left ligament, distal part of the stapes*. Osteoclerosis of the stapes (case 1).

Fig. 4. The layer of the labryl thin capsule is round the perine semicircular canal. A narrow endosteal layer and a well-marked endochondral layer. Numerous medullary spaces and cartilage remnant of the immature bony tissue (case 1).

centrally around the cochlear lumen and a somewhat thicker more continuous layer peripherally. Between these two layers there were bone marrow spaces. The ground substance consisted of few irregularly arranged fibres. Osteocytes were of irregular shape and arrangement. The amount of cartilaginous tissue was relatively increased (Fig. 2).

The periosteal capsule was poorly developed consisting of a cellular web-like bony tissue of a somewhat coarser fibrillar structure than the endochondral layer. In several places the cells were large with vesicular nuclei.

However there was a gradual transition between the peripheral part of the endochondral layer and the periosteal layer the latter showing a denser fibrillar structure.

The cellular part of the labyrinthine capsule showed approximately the same appearance. The endosteal layer was poorly developed and the endochondral layer here too, divided into a less continuous layer centrally and a thicker continuous layer peripherally—with large cartilaginous islets, especially around the fissula ante fenestram (Fig. 3). In this part too the periosteal layer was thin.

Similar changes were found in the capsule surrounding the semicircular canal (Fig. 4) with a faint, faint normal developed endosteal layer. The endochondral layer was of the same structure as in other areas of the labyrinthine capsule.

#### Case 2

Both temporal bones from a 77 year-old man. Clinical diagnosis: Osteogenesis imperfecta tarda.

In the labyrinthine capsule on both sides severe abnormalities, and two types of abnormal tissue could be distinguished (Fig. 5).

Primarily pronounced osteoclerotic changes, about threequarters of the

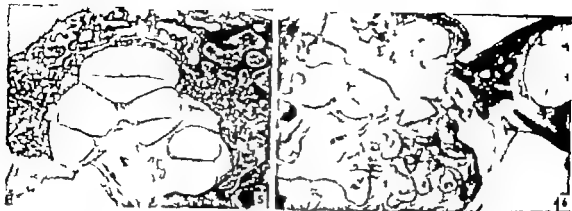


FIG. 5 Part of the labyrinthine capsule with otosclerosis and osteogenesis imperfecta tarda (case 2)

FIG. 6 Oval window. The otosclerosis has encroached upon the footplate of the stapes. Adjacent to the vestibule also osteogenesis imperfecta tarda (case 2)



FIG. 7 Osteogenesis imperfecta tarda in close relation to the cochlea. In the right side of the picture otosclerotic tissue (case 2)

FIG. 8 Sharp borderline between otosclerotic tissue on the left and osteogenesis imperfecta tarda on the right side of the picture (case 2)

total labyrinthine capsule having been converted into tissue of otosclerotic type. In most sites the process was highly active, there being ample vascularized connective tissue with osteoclastic as well as osteoblastic activity in the marrow space. In other sites the activity was less marked; in a few sites the process was "outburnt", leaving empty marrow spaces and bony tissue of low vitality showing only scattered degenerated osteocytes. In the oval window the otosclerosis on both sides had encroached upon the footplate and entailed ankylosis of the stapes (Fig. 6).

Otosclerotic changes were most pronounced in the cochlear part of the labyrinth, gradually losing themselves in the vestibular part.

The other type of tissue changes in the labyrinthine capsule affected the normal bony tissue. In close relation, especially to the lateral border of the basal cochlear turn (Fig. 5) but also around the utricle and saccule there was bony tissue showing the changes of osteogenesis imperfecta tarda.

In the bony cochlear capsule the endosteal bony layer was thin but of normal appearance.

In large areas the endochondral layer (Fig. 7) was built up of very dense, cellular bony tissue, where the normal lamellar bony tissue was replaced by homogeneous bony tissue with cartilage remnants. The borderlines between this tissue, the normal bony tissue, and the otosclerotic tissue were sharp (Fig. 8).

Thus, the normal bony tissue showed quantitative changes, in the form of appreciable increase in the relative amount of connective tissue.

### DISCUSSION

As pointed out by Altmann & Kornfeld (1967) it has not yet been proved that otosclerosis and osteogenesis imperfecta have a common aetiology in spite of histological similarities—viz. the immature bone matrix which in congenital osteogenesis imperfecta is highly reminiscent of the blue mantle of otosclerosis.

Our findings support Altmann & Kornfeld's view that where otosclerosis and osteogenesis imperfecta co-exist the two conditions may be clearly distinguished histologically. In our first case the histological finding characteristic of congenital osteogenesis imperfecta, was an important component in the endochondral layer of the labyrinthine capsule. In the second case there was a distinct histological difference between the otosclerotic tissue and the tissue affected with osteogenesis imperfecta. It cannot be decided whether the otosclerotic process was due to the generalized skeletal disease or whether the simultaneous presence of otosclerosis and osteogenesis imperfecta was a chance coincidence. However there is no histological resemblance between the diffuse skeletal changes in osteogenesis imperfecta and the changes in the otosclerotic focus.

Clinically the two diseases often co-exist. Fowler's study (1949) for instance supports the view of Wullstein-Hall who found that 89% of patients with fixation of the stapes have abnormally blue sclerae. Both diseases are hereditary and more common in women than in men. Similarly 40–60% of patients with osteogenesis imperfecta have fixation of the stapes and blue sclerae.

Biochemical and histochemical studies (Chevance *et al* 1962, Chevance, 1964, Arslan & Ricci, 1963) have thrown new light upon the relationship between the two diseases. As already mentioned, Simon Hall *et al* believe that both are due to a common genetic anomaly in the osteoblast, resulting in the immature bony matrix seen particularly in osteogenesis imperfecta. Chevance's studies reveal that the alteration in the enzymic activity in the osteocyte is the primary factor in the development of an otosclerotic focus.

Through 3 years Chevance (1965) studied about 2500 operative specimens from patients with fixation of the stapes. Of these, 500 were studied histologically as well as histochemically. Only eight of the specimens were

derived from patients with osteogenesis imperfecta. As decisive differences Chevance emphasized that the histochemical studies of osteogenesis imperfecta did not reveal —SH groups and that osteoclasts and osteoblasts were seldom seen at the oval window. Furthermore Chevance never observed blue mantles in osteogenesis imperfecta whereas "microfractures" were common.

Aralan & Ricci (1963) consider otosclerosis as a local manifestation of a generalized connective tissue disease involving alterations in the ground substance of the supporting tissue. Vyzontsis (1956), Bentzen (1961) and Stadler (1961) findings of histological changes in the connective tissue of skin biopsies from patients with otosclerosis and from patients with osteogenesis imperfecta point into the same direction. Solfer *et al.* (1965) have found an increased lactate dehydrogenase activity in the venous wall from otosclerotics compared with normals, and this too indicates a generalized connective tissue disorder. Altmann & Kornfeld (1967) believe that the reason why uniform histochemical changes, in the form of an increased quantity of acid mucopolysaccharides and fewer fibrils, are found in the two diseases, is the morphological similarity between blue mantles of otosclerosis and the immature bone matrix of osteogenesis imperfecta.

In other words, opinions are divided and as yet it is not possible on the basis of the studies performed so far to draw any conclusion about a common aetiology of otosclerosis and osteogenesis imperfecta.

### RÉSUMÉ

La possibilité d'une aetiology commune entre otospongiose et osteogenesis imperfecta est discutée. Les différences de l'histopathologie dans deux os temporales, un avec osteogenesis imperfecta congenita et un avec otospongiose et osteogenesis imperfecta tarda en même temps, sont soulignées. Ce n'est pas possible avec les méthodes de recherche qui sont employées aujourd'hui, de confirmer une aetiology commune entre otospongiose et osteogenesis imperfecta.

### ZUSAMMENFASSUNG

Die Möglichkeit dass Otosklerosis und Osteogenesis Imperfecta eine gemeinsame Ätiologie hat, wird diskutiert. Die histopathologischen Verschiedenheiten in zwei Schläfenbeinen, ein mit Osteogenesis Imperfecta Congenita und ein mit gleichzeitiger Osteogenesis Imperfecta Tarda und Otosklerosis werden betont. Es ist heute mit den neuen Untersuchungsmethoden nicht möglich eine gemeinsame Ätiologie von Otosklerosis und Osteogenesis Imperfecta zu bestätigen.

### REFERENCES

- ALTMANN F. 1962: The temporal bone in osteogenesis imperfecta congenita. *Arch. Otolaryng.* (Chil.) 5, 488.  
 ALTMANN F. and KORNFELD, M. 1967: Osteogenesis Imperfecta and otosclerosis. New findings. *Ann. Otol.* 89, 76.

- AMELIX, M. and RUCK, V. 1962 Histochemical investigation of otosclerosis with special regard to collagen disease. *J Laryng* 77 365
- BARTON, O. 1961 Les anomalies de la peau chez les Otospongieux. Read before the VII International Congr of Otorhinolaryngology Paris, 1961 *Excerpta Med. Int. Congr* 33 (Abstracts, 88 76.)
- CARTER, L. G. 1961 On some histochemical aspects of the otosclerotic focus. *Acta Otolaryng* (Stockh.) 55 175
- 1964 Comparaison des lésions histologiques de la plaque stapediale dans le cours de l'otosclérose et de l'otospongiolite (Maladie de Meniere) et de l'otospongiolite. *Problèmes actuels de l'Oto-Rhino-Laryngologie* 150 Librairie Maloine, Paris.
- CARTER, L. G., CARTER, P. and BOWEN, J. 1962 *Histochémie du foyer Otospongieux* Librairie Arnette, Paris.
- FOULKE, E. P. 1949: The incidence and degree of blue sclera in Otosclerosis and other disorders. *Laryngoscope* 59 466.
- GROTHUES, R. 1928 Die Vererbung der Skleren und Herdenkrankung der Labyrinthkapsel. *Arch. Ohr. Nas. K. H. H. H. H.* 12, 215.
- HALL, J. SIMON, 1961 (Otosclerosis) osteogenesis imperfecta. *Acta Otolaryng* (Stockh.) 53 202
- 1962-63 The pathology of otosclerosis. *Acta Otolaryng* (Stockh.) Suppl. 181
- KLUG, H. R. 1922 Die Labyrinthkapsel bei angeborenen Knochenkrankheiten. *Arch. Ohr. Nas. K. H. H. H. H.* 100 11
- OML, W. R. P. and HALL, J. SIMON, 1962 On the aetiology of otosclerosis. *J Laryng* 72, 841
- RITZ, E. 1922: Osteoparathyrose und Otosklerose. *Z. Ohrenheilk.* 3 263.
- SCHNORR, K. E. 1949 *Osteogenesis Imperfecta*. Thesis. Universitätsverlag, Aarhus.
- SCHNORR, K. E., ALTMAN, P. E., EDA, G. and HOLMSTROM, C. H. 1965 Biochemical studies of otosclerosis. III Lactic dehydrogenase in tissue. *Arch. Otolaryng* (Chic.) 82, 516.
- STANLEY, P. 1961 Examens histologiques de la peau du syndrome de Van der Hoeve. Read before the VII International Congr of Otorhinolaryngology, Paris 1961 *Excerpta Med. Int. Congr* 33 (Abstracts 33/124)
- WISSE, M. 1928 Osteogenesis imperfecta congenita der Labyrinthkapsel. *Arch. Ohrenheilk* 23, 245
- 1930 Z. Theorie der sporadischen Otosklerose. *J. h. Ohrenheilk* 28, 52.
- WILSON, H., OSMAN, R. P. and HALL, J. SIMON, 1960 V. der Hoeve's syndrome in mother and daughter. *J Laryng* 70 67
- WILSON, E. 1964 Über Veränderungen der Hautfalten bei Otosklerosen. *Z. Laryng Rhinol Otol* 35 182.

Dept. of Otolaryngology  
Rigshospitalet i Blegdamsvej  
Copenhagen Denmark

## DISCUSSION

A. Lasker: The middle layer of the labyrinthine capsule is from the earliest days of life only partially replaced by endochondral bone s.c. globuli over, while the major portion is replaced by bone marrow which is then compacted by apposition of endosteal convoluted bone and the mesenchymal bone-forming cells are able to form bone directly. In a number of cases it forms a secondary chondroid type of cartilage which may be replaced by endochondral globuli over. Osteoclastic resorption and lacunar erosions with accumulations of



bone forming mesenchymal cells within the eroded lacunar areas are still present. Otosclerotic bone within its areas is a new bone formation within fibrous tissue, while the a.v. invasive bone spreads like a wave into the old capsular tissue with confluence of the perivascular bone areas. Blue mantles represent an attempt by the uninvolved portion of the capsule to repolymerize mucopolysaccharide matrix which loses its mineral by a process of halisteresis rapidly along physiological channels as remodeling rate about the vessels with increased mineralisation osteoclastic resorption and accumulation of bone forming mesenchymal cells for the extensive degeneration in adjoining areas. As for the factors being responsible for the process in question it may be quoted that hereditary constitutional factors, thermal, inflammatory and mechanical (acoustic) ones, and irradiation have been suggested by Kristensen to initiate a preparedness in developing the otosclerotic process by inducing activation of dormant embryonic or a presence of repair upon a microscopic focus in aseptic necrotic area caused by irradiation

*H C Andersen* Mr Breilau shows microscopy findings in three patients suffering from osteogenesis and otosclerosis. In one typical otosclerotic changes were found in the stapes. In two other cases there was no sign of otosclerosis, but changes resembling osteogenesis imperfecta. It is concluded that conductive deafness in osteogenesis imperfecta can be produced by otosclerosis or by osteogenesis in itself

*J G Hall* First I think it is appropriate to recall the findings which our former professor and member of this Collegium Odd Ophelm, published in the Acta no 85 this year. During the period 1960-65 Ophelm in five cases of osteogenesis found epithelium lunae cavities in the capitulum stapediale and structurally altered crurae with a fibrous degeneration

Mr Röhr and myself in the same number of the Acta reported a case in which histological examination of the stapes was performed. No otosclerotic foci were found the stapediale joints were normal, but the crurae were thin, frayed, in some places thread like with only a fibrous connection to the footplate

Today we hear that histological changes of this type are found in connection with otosclerotic foci just like others have reported before. I think it is reasonable to conclude that we do not have enough support for the presumption of a basic and common etiology in these diseases. We are still at a loss concerning the explanation of these coincidences.

*Balslev Jørgensen* (Reply) to Mr Laskiewicz- We want to point out that in our studies of temporal bones we have found the so-called "blue mantles" in normal bones as well as in different pathological conditions. Consequently we do not consider blue mantles as a sign of latent or incipient otosclerosis.

To Mr Andersen Your slides seem to support our view that otosclerosis and osteogenesis imperfecta are two clearly distinguishable conditions from the histological point of view

To Mr Hall Your comments point in the same direction. We apologize that we forgot to mention Professor Oppheim's important paper which appeared recently in the Acta.

## QUELQUES RÉSULTATS DES EXAMENS OTOLOGIQUES ET AUDIOLOGIQUES DES INDIENS SUD-AMÉRICAINS

J M TATO et J M TATO

*Buenos Aires Argentine*

On a examiné oto- et audiologiquement 11 000 individus, dans différentes zones du Pérou, de la Bolivie et du Paraguay appartenant au groupe d'indiens Andinos ou Pampidos, d'âges 6500 de race pure ou presque et 5000 métis. Quant à l'otosclérose, chez le groupe indien pur on a trouvé 2 cas unilatéraux probables, ce qui donne fait un indice de 0,03%. Chez les métis 0,3%.

Le *Núcleo Latinoamericano para el estudio de la audición y equilibrio* a décidé de promouvoir une étude sur l'audition de la population indienne de l'Amérique Latine.

Une équipe dirigée par J M Tato (p) a été constituée par les docteurs Jaime Flores, comme directeur-assistant, J M Tato (f) Anibal Arabel, Luis Vinates et les audiologistes E. Sarrafi, M. Lauberer et E. T. de Slaria avec la collaboration temporaire selon les lieux, des docteurs R. Porcel, A. Bustillo, Alfredo Padilla en Bolivie D. Tejada et G. del Carpio au Pérou Dra. G. Santos et R. de la Torre au Paraguay.

L'investigation commença en 1961 à Salta, Oran, Argentine, dans le N O près de la frontière bolivienne.

A partir de 1963 elle se poursuivit au Pérou avec l'appui économique d'un fonds N.B.03 006.01-03, de l'Institut National de la Santé, du Département de la Santé de l'Education et de l'Hygiène des Etats-Unis d'Amérique. Tenant compte de l'opinion des autorités de la Santé Publique et des Instituts Indigènes des différents pays, les endroits furent choisis en fonction des possibilités des conditions locales et de l'acceptation par la population d'être examinée.

En voici la liste

Année	Zone et pays	Nombre d'examens
1961	Oran, Salta, Argentine	591
1963	Yunguyo, Pérou	1 338
1963	Pun et Lauca, Pérou	93
1963	Tacna, Pérou	710
1964	Lima, Pérou	1 416
1965	Hacienda Vicos, Pérou	600
1965	Huancabamba, Pérou	950

1965	Asunción, Paraguay	100
1905	Philadelfia, Paraguay	1 818
1906	Siglo XX et Catavie, Bolivie	3 039
1908	Irphachico Bolivie	1 102

Total 11 757

Du total 1378 furent examinés otologiquement, l'examen fonctionnel étant limité aux diapasons, et chez tous les autres (10 379) une audiométrie liminaire aérienne et osseuse fut pratiquée

En cas de suspicion d'otosclérose l'examen fonctionnel fut complété par les épreuves de Gellé, Runge, l'indice de Sullivan et dans les derniers cas par la mesure de l'impédance absolue et relative

Les objectifs de l'étude furent multiples l'un d'eux a été d'établir l'existence de l'otosclérose et si oui, dans quel pourcentage Des questions connexes ont été également étudiées.

La population indigène pure (du moins au plus grand degré) a été de 6 163 pour 4 340 métis (blancs et indiens) Ces chiffres résultent des études statistiques faites par le Département de la Santé Publique de la République Argentine au moyen de machines calculatrices et d'ordinateurs Les âges limites des personnes examinées étaient de 8 et 85 ans Chez les indigènes, nous avons trouvé deux cas probables d'otosclérose unilatérale Chez les métis 5 cas Cela représente

	% d otosc.
Parmi 6163 indiennes pures de 8 à 85 ans	0 03
Parmi 5141 indiennes pures de 20 à 85 ans	0 04
Parmi 4340 métis de 8 à 85 ans	0 11
Parmi 3408 métis de 20 à 85 ans	0 14
Guild parmi 585 temporaux de blancs de 1 à 80 ans	7 25
Guild parmi 518 temporaux de blancs de 6 à 80 ans	8,30
Sercer parmi 200 crânes de blancs	1,25
Cliniquement parmi la population blanche de 0 50 à 1	

Rosen n a pas rencontré d'otosclérose chez les Nègres ni au Sudan ni aux Bermudes S Guild a trouvé dix fois plus de cas d'otosclérose dans la population blanche que dans la population noire de Baltimore Ainsi il existe une grande différence d'apparition de l'affection entre les races

Selon Scheer cette différence serait due à la torsion de la pyramide pétreuse et du labyrinthe qui se manifeste dans les variations de l'angle sphénoïdal qui est différent selon la race

Ceci nous a incités à le mesurer chez les Indiens, étude effectuée par J M Tato (f) grâce à des radiographies de profil et des tomographies sagittales du crâne dans la population actuelle.

En voici les résultats promediels

8 crânes secs de Pampidos argentins	130 7
7 quechuas vivants du Pérou	135 3
10 aymaras vivants du Pérou	134 6

Il a pratiqué des mesures directes de l'angle sphéno-occipital ou de Welker sur 188 crânes secs provenant d'Indiens de Patagonie et du Nord-Ouest, appartenant au Musée Anthropologique National d'Argentine.

Parmi ceux-ci existent des crânes normaux et des crânes déformés selon la tradition.

Les valeurs moyennes furent les suivantes

— Crânes normaux	132 3
— Crânes déformés	
— tabulaire erect	134 6
— tabulaire erect plan frontal	138 3
— tabulaire erect plan labioïde	133 6
— tabulaire oblique	142 6

Selon Sereer ces valeurs seraient pour

— les crânes yougoslaves	117
— les crânes japonais	135
— les crânes des anciens péruviens et boliviens	132

Nous devons souligner la coïncidence entre les valeurs angulaires des crânes des Indiens Sud Américains et des Japonais (selon Sereer) et la concordance avec l'hypothèse de Sereer selon laquelle les Indiens ont un angle beaucoup plus ouvert que les blancs.

Les mesures d'impédance absolue et relative chez les Indiens péruviens et boliviens coïncident avec celles obtenues au Danemark, aux U.S.A. et à Buenos Aires (Argentine)

On rencontre des différences morphologiques dans la forme du pavillon, parmi lesquelles le prolongement de la racine de l'hélix qui divise la conque en deux parties dans 5 % des cas le conduit auditif est droit dans sa paroi antérieure osseuse et dans les crânes préhistoriques du Musée Anthropologique de La Paz (Bolivie) sa paroi antérieure est même concave l'insertion tympanique se fait dans un plan plus oblique vers l'avant ce qui explique l'absence ou la modification du triangle lumineux et le raccourcissement apparent de la paroi postérieure du tympan dû à la perspective. Ces modifications n'ont jamais été décrites à notre connaissance.

Dans des publications antérieures nous avons décrit des cas d'otosclérose chez des Indiens prétendu purs en dehors de notre enquête actuelle. Nous les avons fait examiner par des anthropologistes qui ont conclu qu'il n'y avait aucun doute de métils.

Des rares cas ont été décrits par des collègues boliviens, mais sans confirmation anthropologique.

Dans une des régions (Irapachico, Bolivie) caractérisée par son endogamie

pendant plusieurs siècles où l'incidence d'une maladie héréditaire devrait être plus élevée nous avons examiné 25 % de l'ensemble de la population, soit 1088 sujets sans trouver aucun cas d'otosclérose

### SUMMARY

A group of 11 000 people belonging to the Andido and Pámpido Indian races were examined otologically and audiotologically in different parts of Peru Bolivia and Paraguay About 6500 of these were real Indians and about 5000 were half breeds As regards otosclerosis in the group of pure Indians only two unilateral probable cases were discovered giving therefore an average of 0.03% For the half-breeds it was also 0.3%

### ZUSAMMENFASSUNG

Wir untersuchten otologisch und audiotologisch 11 000 Indians aus der Gruppe der Andiden oder Pampiden aus verschiedenen Regionen Perus, Boliviens und Paraguays. Davon waren 6500 reinrassig oder beinahe und 5000 Mischlinge Bezüglich Otosklerose fanden wir unter den reinrassigen Individuen 2 wahrscheinlich einseitige Fälle was einen Index von 0.03% ausmacht Unter den Mischlingen betrug er 0.3%

*As néaga 235*

*Buenos Aires Argentina*

## VISUAL REINFORCEMENT AUDIOMETRY

G. LIDÉN and A. KANAKUVEN

*From the Department of Otology and Audiology Sahlgrenska Hospital,  
University of Göteborg Göteborg Sweden*

Visual reinforcement audiometry is a new technique for testing hearing in very young children. This method is a modification and simplification of conditioned orientation reflex audiometry by Suzuki & Ogiba. Instead of using the sound localization or eye deviation reflex as a response criterion we are using a no direction technique and accept four different types of responses to the tone stimulation reflexive behaviour in obligatory orientation and spontaneous responses. Hearing levels of normal hearing children of different age groups tested with both methods are presented.

The early identification and management of the auditorily handicapped child has been shown to be of paramount importance for the general development of the child as well as for the development of his speech and language. Exact knowledge of the auditory function thus is necessary for planning a training program for children with communicative disorders.

The neonatal auditory test procedures described by Ewing & Ewing (1947) Wedenberg (1956) Hardy *et al* (1959) and Downs & Sterritt (1964) have proven very helpful in the early identification of deafness. From the audiological and educational point of view however these methods are too crude and do not provide us with sufficient information concerning the child's threshold sensitivity. In fact, until recently the period from birth up to 2 / or 3 years of age, when play audiometry (Barr 1955) can be used, has remained baffling with regard to threshold audiometry.

Suzuki & Ogiba (1960) introduced conditioned orientation reflex audiometry (CORA) as a way out of these difficulties. According to their report the binaural tone thresholds could successfully be determined with more than 85% of 174 children between 1-3 years of age. Their method is simple. The child sits in front of the equipment. On each side of its front panel there is a speaker fed by a pure-tone audiometer. Above each speaker is a semitransparent doll which can be illuminated. The distance between the speakers is about 70 cm. The eye orientation reflex in response to pure tones is reinforced by illuminating the doll on different sides of the panel and the conditioning is made by pure tone. After a few trials when the child is conditioned he usually looks immediately towards the doll in response to the tone stimulus.

This study was supported by the Swedish Medical Research Council

We have used Suzuki & Ogiba's method and found it very useful in normal hearing young children but too difficult for many hard of hearing children. Reddell & Calvert (1967) seem to be of the same opinion, according to a recent report. Another disadvantage with their method is that it achieves only a binaural threshold. Therefore we modified the equipment and changed the testing technique. Instead of using the sound localization or eye deviation reflex as a response criterion we used a nondirectional technique and accepted four different types of responses to the tone stimulation: reflexive behavior, investigatory, orientation and spontaneous responses. These types of response will be discussed below. We have called this modification *visual reinforcement audiometry* (VRA).

## PRESENT INVESTIGATION

### *Purpose*

The purpose of our investigation was to answer the following questions concerning visual reinforcement audiometry (VRA):

1. Is VRA a successful technique for eliciting responses to auditory stimulation in normal and hard of hearing preschool children?
2. How does the hearing level in normal preschool children compare with normal hearing for adults when using VRA?
3. How do the responses vary in different children of different age groups when using VRA?
4. How does the efficiency of testing the hearing of preschool children using visual reinforcement audiometry compare with that of conditioned orientation reflex audiometry?
5. How long during a test session can we maintain the interest of the child?

### *Subjects*

#### *Normal hearing children*

Two groups of subjects were used in this study. One group consisted of 120 children with normal hearing, while the other contained 935 investigations on children with varying degree of hearing impairment. These two groups shall be described in greater detail. The parents of 148 supposedly healthy children who were visiting the two well baby clinics located closest to the hospital for routine check-ups were asked to participate in the hearing test. The children were selected at random by choosing every third child belonging to the following age groups: 3 months to 1 year, 1 to 2 years, 2 to 3 years, 3 to 4 years, 4 to 5 years or 5 to 6 years. Clinical examinations revealed 17 children with some middle ear involvement, five with sensorineural hearing loss and six with mental retardation. These 28 children were excluded from this study. The remaining 120 children were distributed evenly in the abovementioned six age groups.

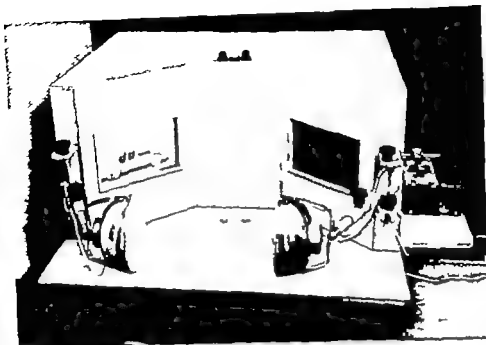


Fig. 1 Anterior view of the Visual Reinforcement (VRA) audiometer

### *Hard-of-hearing children*

Since 1964 visual reinforcement audiometry has been used routinely in our laboratory. In order to answer some of the above mentioned questions we have analyzed our records on children with suspected or verified hearing loss up to the end of 1966. Altogether 935 investigations were performed.

### *Equipment*

Instead of using illuminating dolls as visual reinforcement for the orientation eye reflex, we alternately project slides on two frosted glass windows located on either side of the curved front panel of the box shaped apparatus (Fig. 1). The slides are specially selected by our preschool audio-educators to maintain the interest of the child. The switching of the slides from right to left and vice versa is arranged electronically with the help of mirrors (Fig. 2). The switch board is placed on the audiometer. The loudspeakers are mounted on two separate movable and adjustable arms making it possible to present the tones with an azimuth of 90 degrees on each side. The audiometer provides the following test frequencies: 0.25, 0.5, 1, 2, 3 and 4 kHz. In this investigation the tones have been presented steady but later we also included frequency modulation (warble tone). The intensity range is -10 dB to 110 dB regulatory in 5 dB steps. The maximum hearing level is 90 dB for 250 Hz, 100 dB for 500 Hz and 110 for the others. The audiometer with the loudspeaker was calibrated in an anechoic room on



We have used Suzuki & Ogilby's method and found it very useful in normal hearing young children but too difficult for many hard of hearing children. Reddell & Calvert (1967) seem to be of the same opinion, according to a recent report. Another disadvantage with their method is that it achieves only a binaural threshold. Therefore we modified the equipment and changed the testing technique. Instead of using the sound localization or eye deviation reflex as a response criterion we used a nondirectional technique and accepted four different types of responses to the tone stimulation: reflexive behavior, investigatory, orientation and spontaneous responses. These types of response will be discussed below. We have called this modification *visual reinforcement audiometry* (VRA).

## PRESENT INVESTIGATION

### *Purpose*

The purpose of our investigation was to answer the following questions concerning visual reinforcement audiometry (VRA):

- 1 Is VRA a successful technique for eliciting responses to auditory stimulation in normal and hard of hearing preschool children?
- 2 How does the hearing level in normal preschool children compare with normal hearing for adults when using VRA?
- 3 How do the responses vary in different children of different age groups when using VRA?
- 4 How does the efficiency of testing the hearing of preschool children using visual reinforcement audiometry compare with that of conditioned orientation reflex audiometry?
- 5 How long during a test session can we maintain the interest of the child?

### *Subjects*

#### *Normal hearing children*

Two groups of subjects were used in this study. One group consisted of 120 children with normal hearing, while the other contained 935 investigations on children with varying degree of hearing impairment. These two groups shall be described in greater detail. The parents of 148 supposedly healthy children who were visiting the two well baby clinics located closest to the hospital for routine check-ups were asked to participate in the hearing test. The children were selected at random by choosing every third child belonging to the following age groups: 3 months to 1 year, 1 to 2 years, 2 to 3 years, 3 to 4 years, 4 to 5 years or 5 to 6 years. Clinical examinations revealed 17 children with some middle ear involvement, five with sensorineural hearing loss and six with mental retardation. These 28 children were excluded from this study. The remaining 120 children were distributed evenly in the abovementioned six age groups.

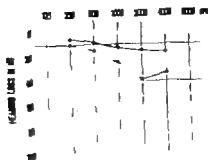


FIG. 3 (a) The head shadow effect measured with an artificial head in the sound field of the VR-a audiometer. The sound level recorded from far ear (solid line) is plotted relative to that of near ear (0 dB). (b) The "head shadow effect" with an artificial ear. The sound level recorded from far ear (dotted line) is plotted relative to that of near ear (0 dB).

greater deviation from this pattern should arouse suspicion of partial or complete monaural hearing loss. According to Nordlund (1962) the head shadow may reduce the intensity of the stimulus in an anechoic room in the far ear by about 5 dB for the frequencies 250 and 500 Hz and between 10 and 25 dB for 1000–4000 Hz. In other words if we tested the threshold sensitivity on the left side of a child who was deaf on that side we could expect to get a considerable drop of the tone threshold only for the higher frequencies.

In order to find out more about the head shadow effect in the actual testing situation we used an artificial head as described by Nordlund & Liden (1963) as a substitute for the child's head. In Fig. 3a the intensity of the tones in the far ear are displayed relative to the near ear. As can be seen from this figure the head shadow reduces the intensity in the far ear mainly for 2000 and 4000 Hz. By inserting an ear plug in the far ear the head shadow effect increases considerably (Fig. 3b). Using the VR audiometer this effect can be utilized for detecting monaural deafness in small children.

The artificial head was also used to elucidate the change in tone intensity at near and far ear induced by the normal movement of a child's head during test session. A movement of the artificial head up to 5 cm in all direction including 20° rotation from its original place was used to simulate the movement of the child's head. The recordings showed as an average a variation of 3 dB in sound pressure level with 10 dB as maximum variation at 4000 Hz.

Primarily our apparatus was designed for measuring the monaural sound field threshold for pure tones. However by switching the tone stimulus and the picture from side to side it could also be used for determining the binaural tone threshold with the conditioned orientation eye reflex according to Suzuki & Ogiba. By some other simple arrangements the equipment

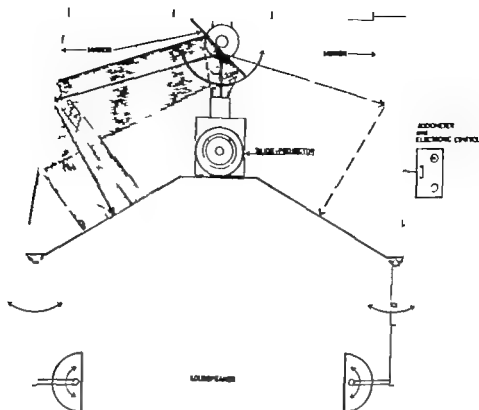


FIG. 2 Block diagram of the VR-audiometer

10 normal subjects whose threshold hearing level in both ears was in the range of  $\pm 5$  dB re ISO standard threshold of hearing for pure tones with headphones. The loudspeakers were placed 15 cm lateral of the subject's ears, i.e. at 90 degrees azimuth. The ear not under test was blocked with an insert ear plug and covered with an external muff. The thresholds were determined by the method of limits. The mean values of the attenuator settings for both ears were averaged. The sound pressure levels corresponding to the attenuator settings for the minimum audible field were read when the subject was not present. These values served as reference level for the hearing tests on children (Table 1). The actual testing of the children was performed in a sound proof room. The calibration was finally rechecked on the same subjects who served in the anechoic room.

The placement of the loudspeaker lateral of the child's head makes it possible to utilize the head shadow effect in separating right and left ears tone thresholds in spite of the absence of masking. In normal subjects we can expect rather identical sound field curves for both ears and thus any

TABLE 1 Mean monaural minimum audible sound field in sound pressure re 0.0002 dyne/cm<sup>2</sup> for 10 normal adults with the VR-audiometer

250 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
19	7	1	-3	-3.5



FIG. 4.

FIG. 4 Test-retest variations in threshold sensitivity. Eight hard-of-hearing subjects (16 ears) between 1 and 3 years of age. Each dot represents the difference between first and second test in dB for each subject.



FIG. 5.

FIG. 5 Comparison between thresholds obtained with VRA and earphone audiometry. Fifteen hard-of-hearing subjects (26 ears) at 1000 Hz between 8 months and 2 1/2 years of age. Each dot represents the difference between first and second test in dB for each subject.

Our six age groups consisting of 120 otologically normal children could all successfully be tested in one session using visual reinforcement audiometry.

However the number of normal hearing children in these groups was rather small. Therefore we included an analysis of 935 investigations on children with suspected or verified hearing loss. The VRA technique was found to be very useful in that all children but 10 of the 935 could successfully be tested.

The reliability of the threshold measurements judged by test retest results of VRA on eight hard-of-hearing children (16 ears) in the age group of 1 1/2 to 3 years old was good as can be seen in Fig. 4. The retest was made about one month later. Another way of checking the reliability of VRA is to compare the threshold results obtained with VRA and ear phone audiometry. Such a comparison was made on 15 hard-of-hearing children (26 ears at 1000 Hz) in the age group 8 months to 2 1/2 years old. The two measurements were made in the same test session. The differences in thresholds are plotted in Fig. 5.

2. How does the hearing level in normal pre-school children compare with normal hearing children and adults when using VRA?

The mean threshold curves for the different age groups measured with VRA are shown in Fig. 6. As can be seen the children's threshold fall short of normal hearing adults. This does not mean that the auditory organ in children is functioning worse than in adults but that children need some

could also be used for eliciting the acoustopalpebral reflex, for the arousal test for hearing aid evaluation in young children and as a peep show test (Dix & Hallpike 1947)

### *Procedure*

As a rule the hearing session started with (I) visual reinforcement audiometry and finished with (II) conditioned orientation reflex audiometry

#### *I Visual reinforcement audiometry*

The child was trained to respond to a tone coming from the same side as the subsequent picture. No attention was paid to the tone localization and concomitant eye movement. The child was never instructed to respond in a particular way and neither was he expected to give a specific type of response. Consequently a broad variety of responses could be observed. These are discussed below under results.

Children under 3 years of age were seated in the lap of the mother in front of the apparatus and were permitted to play with toys. The testing procedure was not explained.

Children over 3 years of age as a rule were not given any toys to play with but were told that they were going to hear a sound in one of the speakers, and after that see a picture.

The session started with a 500 Hz tone presented to the right loudspeaker at 30–40 dB above estimated threshold. If the child below 3 years old responded he generally stopped playing for a moment and looked at the loudspeaker or the audiologist who pointed at the right window and projected a slide there. The play with toys started over again and a new set of tones and pictures were delivered on the same side a couple of times. By gradually reducing the intensity and watching the response of the child it was possible to obtain a tone threshold for the right ear. The same procedure was then repeated for the left ear with the exception that the left speaker and the left window were utilized for this measurement.

#### *II Conditioned orientation reflex audiometry*

The child was trained to the same combination of tone and picture but with the change that the sides of presentation of stimuli were randomly alternated. The criterion for a correct response to the tone stimulus was now the immediate look at the picture or the loudspeaker on the same side as the presented tone. Thus this method corresponds closely to that used by Suzuki & Ogiba.

### RESULTS

1 Is visual reinforcement audiometry a successful technique for eliciting responses to auditory stimulations in normal and hard of hearing pre-school children?

(c) Some children learned very quickly that every tone was followed by a picture. In this group no expectation on the face of the child could be noted. No other evidence of reflex response than the immediate look towards the window could be found. We have called this reaction an orientation response.

(d) We have designated the fourth type as spontaneous response. This type of response is most highly developed. This means that the child reports directly that he heard the tone and that he immediately understands what is going on. The spontaneous response could be expressed in many different ways from pointing or looking at the window to raising the finger imitating the tone, just smiling, nodding or saying "now".

4. How does the efficiency of testing the hearing of pre school children using visual reinforcement audiometry compare with that of conditioned orientation reflex audiometry?

For the otologically normal children the binaural tone threshold as judged by conditioned orientation reflex audiometry are shown in Fig. 7. The results look very similar to those of VRA (Fig. 6). However a detailed comparison between the threshold curves for the age group 1 and 4 years old determined with both methods demonstrated certain differences. In the youngest group both methods seemed to give similar thresholds. The group 4 years old on the other hand did considerably better with the VRA technique than with COR audiometry.

5. For how long a time during the test session could the interest of the child be maintained using these methods?

The 15 minutes time reserved for every child was long enough for determining the tone threshold with the use of both techniques. The visual reinforcement audiometry involved five correct responses to a minimum audible sound field on each side. The binaural threshold assessed with the conditioned orientation reflex audiometry needed five correct responses to a minimum sound field.

The reinforcements with the different slides kept the child's motivation in the test situation high. However it was easy to see that the children between 1 and 2 years of age did best during the first 5 minutes, and the older ones during the first 10 minutes.

## DISCUSSION

Any response of the child to a sound stimulus was accepted with the visual reinforcement audiometry. This made it easier to get a threshold response. On the other hand, the inherent difficulties of the method were also evident. The evaluation of the responses in terms of true responses depended very much on the qualifications of the audiologist. Erroneous conclusion based on unconscious changes of facial expression of the audiologist could influence the child to respond to these gestures and not to the sound.

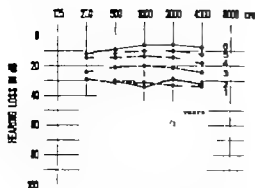


FIG. 6

FIG. 6 Mean monaural sound field threshold curves for different age groups obtained with VRA.

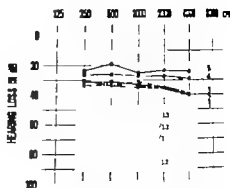


FIG. 7

FIG. 7 Conditioned orientation reflex audiometry. Mean binaural threshold curves for different age groups.

what louder sounds before they respond. With other words the mean threshold curves reveal hearing nearer that of adults as the children grow older indicating maturation of response.

3. How do the responses vary in different children of different age groups when using VRA?

The aim of the visual reinforcement was of course, to make the rather meaningless pure tones more interesting to the children. The response of the children in the two youngest age groups was very much dependent on their ability to associate the tone stimulus with the picture. The fact that the child was not expected to give a specific type of response was advantageous, and he became very cooperative and active even though the audiologist was a stranger to him and it was the very first time he had his hearing tested. Because of the limited time we expected the child to maintain the interest and motivation for the test only a few pairings of auditory stimulus and visual reinforcement were presented for training purpose. Consequently the children gave a variety of different responses. The four main types of responses encountered are listed below.

(a) The most primitive type of response could be classified as *reflexive behavior*. A first sign that the tone had been heard was that the intense and often somewhat expectant expression on the child's face changed. A variety of movements of the shoulders or head were accepted as well as facial expressions such as wrinkling of the forehead, widening of the eyes, jerks in the lips or change of rate of eye blinking. The reflexive type of response was mainly found in the first age group (below 1 year).

(b) The youngest children did not immediately understand the connection between the tone and the picture but they gradually learned. The first step to comprehension was a look towards the loudspeaker. Next they associated the tone with the audiologist and faced her. These types of "What is it" reflex we have called *investigatory responses*.

(c) Some children learned very quickly that every tone was followed by a picture. In this group no expectation on the face of the child could be noted. No other evidence of reflex response than the immediate look towards the window could be found. We have called this reaction an orientation response.

(d) We have designated the fourth type as spontaneous response. This type of response is most highly developed. This means that the child reports directly that he heard the tone and that he immediately understands what is going on. The spontaneous response could be expressed in many different ways from pointing or looking at the window to raising the finger imitating the tone just smiling, nodding or saying "now".

4. How does the efficiency of testing the hearing of pre-school children using visual reinforcement audiometry compare with that of conditioned orientation reflex audiometry?

For the otologically normal children the binaural tone threshold as judged by conditioned orientation reflex audiometry are shown in Fig. 7. The results look very similar to those of VRA (Fig. 6). However a detailed comparison between the threshold curves for the age group 1 and 4 years old determined with both methods demonstrated certain differences. In the youngest group both methods seemed to give similar thresholds. The group 4 years old on the other hand did considerably better with the VRA technique than with COR audiometry.

5. For how long a time during the test session could the interest of the child be maintained using these methods?

The 15 minutes time reserved for every child was long enough for determining the tone threshold with the use of both techniques. The visual reinforcement audiometry involved five correct responses to a minimum audible sound field on each side. The binaural threshold assessed with the conditioned orientation reflex audiometry needed five correct responses to a minimum sound field.

The reinforcements with the different slides kept the child's motivation in the test situation high. However it was easy to see that the children between 1 and 2 years of age did best during the first 5 minutes, and the older ones during the first 10 minutes.

## DISCUSSION

Any response of the child to a sound stimulus was accepted with the visual reinforcement audiometry. This made it easier to get a threshold response. On the other hand, the inherent difficulties of the method were too evident. The evaluation of the responses in terms of true responses depended very much on the qualifications of the audiologist. Erroneous conclusions based on unconscious changes of facial expression of the audiologist could influence the child to respond to these gestures and not to the sound.



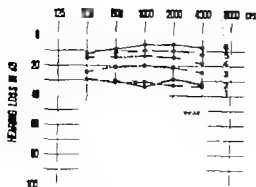


FIG 6

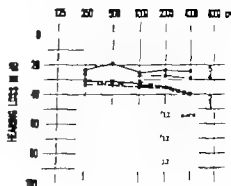


FIG 7

FIG 6 Mean monaural sound field threshold curves for different age groups obtained with VRA

FIG. 7 Conditioned orientation reflex audiometry. Mean binaural threshold curves for different age groups.

what louder sounds before they respond. With other words the mean threshold curves reveal hearing nearer that of adults as the children grow older indicating maturation of response.

3. How do the responses vary in different children of different age groups when using VRA?

The aim of the visual reinforcement was of course, to make the rather meaningless pure tones more interesting to the children. The response of the children in the two youngest age groups was very much dependent on their ability to associate the tone stimulus with the picture. The fact that the child was not expected to give a specific type of response was advantageous, and he became very cooperative and active, even though the audiologist was a stranger to him and it was the very first time he had his hearing tested. Because of the limited time we expected the child to maintain the interest and motivation for the test only a few pairings of auditory stimulus and visual reinforcement were presented for training purpose. Consequently the children gave a variety of different responses. The four main types of responses encountered are listed below.

(a) The most primitive type of response could be classified as *reflexive behavior*. A first sign that the tone had been heard was that the intense and often somewhat expectant expression on the child's face changed. A variety of movements of the shoulders or head were accepted as well as facial expressions such as wrinkling of the forehead, widening of the eyes, jerks in the lips or change of rate of eye blinking. The reflexive type of response was mainly found in the first age group (below 1 year).

(b) The youngest children did not immediately understand the connection between the tone and the picture but they gradually learned. The first step to comprehension was a look towards the loudspeaker. Next they associated the tone with the audiologist and faced her. These types of "What is it" reflex we have called *investigatory responses*.

positivt r Befunde haben wir anstatt uns des Schalllokalisationsvermögens oder häufigen Seitenblickreflexes zu bedienen, eine nicht richtungsbedingte Technik angewandt bei der wir vier verschiedene Typen spontaner durch einen Prüfling erzeugten, Reaktionen akzeptieren.

## REFERENCES

- BARR, B., 1936: Pure tone audiometry for pre-school children. *Acta Otolaryng* (Stockh.) 3 391.
- DIX, M. R., and HALL, G. E., 1947: The peep show. New technique for pure-tone audiometry in young children. *Brit. Med. J.* 2, 710.
- DOWNS, M. P., and STEWART, G., 1964: Identification audiometry for neonates. *J. Aud. Res.* 4 69.
- HALL, T., and EYRE, A., 1947: *Opportunity and the deaf child*. C. Iv of London Press.
- HART, J. DOUGHERTY, A., and HART, W., 1949: Hearing responses and audiologic screening in infants. *J. Pediat.* 55, 382.
- KRICK, G. A., 1961: *Conditioning and learning* (ed. E. R. Hilgard and D. G. Marquis) 1940. H. Appleton-Century-Crofts, Inc., New York.
- LODGE, G. and KILPATRICK, A., 1963: Visual reinforcement audiometry. *Arch. Otolaryng* (Chic.) 1 press.
- LOOMIS, L. L., 1965: Use of the slide show audiometric technique with mentally retarded children. *Exceptional Children*, 32, 93.
- 1966: Behavioral audiometry tested as an operant procedure. *J. Speech Hear. Dis.* 31 128.
- KONDRAT, B., 1962: A gale localization. *Act. Otolaryng* (Stockh.) 53 405.
- KONDRAT, B. and LODGE, G., 1963: A artificial head. *Act. Otolaryng* (Stockh.) 56 493.
- NEDELL, R. C., and CAVERT, D. R., 1967: Conditioned audio-visual response audiometry. *Proc. inf. conf. and symp. f. h. deaf* 1 302.
- SUTCLIFF, T. and OAMA, T., 1966: A technique of pure tone audiometry for children under 3 years of age. *Rev. Laryng.* 81 23.
- 1961: Conditioned orient reflex audiometry. *Arch. Otolaryng* (Chic.) 74 192.
- WERNER, E., 1938: Auditory test on newborn infants. *Act. Otolaryng* (Stockh.) 48 416.

Dept. of Otolaryngology  
Sälgrenska Hospital  
Göteborg Sweden

## DISCUSSION

*A Lasker:* The peep-show was designed in 1947 by Mrs M. R. Dix to overcome the great practical difficulties of obtaining rapidly a reliable pure tone audiogram in young children. This method of testing hearing in children is still one in which there are few facilities for repeated observation by a number of audiologists. Trenquer St. Isten, Wishart (Toronto), Mitrowsky (Warsaw), Palta, Lang, Urban, and Csanyi (Hungary) have stated that Mrs Dix's idea in peep-show testing of hearing in children is of great practical value having a particular place in the armory of audiologists.

J. E. Bordignon: The most important points emphasized by M. Lidén is that he is not actually obtaining thresholds for sound stimuli in the young child. It takes considerable intensity of stimulus in such children to obtain their attention, and such a highly requirement diminishes as the child grows. Such

It was also easy to overlook a child's way of responding if one expected the child to respond in a certain way.

The thresholds obtained for the older children by the COR-technique seemed to be influenced in a favorable direction by practice. In the younger group no such factor was noticed. It was quite evident that the COR technique with the tones alternately presented in the right and left speaker gave poorer thresholds than with the more simple VR method. For some children the COR technique was too difficult and as a result some stopped giving responses at all. The children became tired quickly and needed retraining or reinstruction with the COR technique more than with VR.

VR should not be confused with the slide show audiometric technique described by Lloyd (1905) nor with the peep show by Dix & Hallpike (1947). Although there are some similarities in the equipment the fundamental difference is found in the way the child is responding to the stimulus. In VR the child is not instructed to respond in a particular way. Thus no active co-operation of the child is required as in the other methods.

### CONCLUSION

Visual reinforcement audiometry has proven to be a successful technique for eliciting responses to auditory stimulation. Hard-of-hearing children could reliably be tested from about 6-8 months old. Compared to adults the youngest children needed somewhat louder sounds before they responded. With other words the mean threshold curves revealed hearing nearer that of adults as the children grew older indicating maturation of response.

Conditioned orientation reflex audiometry also appeared to be a useful technique for measuring hearing of pre-school children. This test however was more difficult than VR. The sound localization task proved too complicated for many children and louder sounds were often required for a correct response. This was particularly evident in the older age groups in which COR audiometry gave poorer thresholds than VR.

### RÉSUMÉ

*Landométrie tonale avec renforcement visuel est une modification et une simplification du réflexe conditionné d'orientation. Au lieu d'employer la capacité de localisation du son ou le réflexe de déviation de la tête comme critère de réponse nous employons une technique non directionnelle et acceptons quatre différents types de réponses spontanées.*

### ZUSAMMENFASSUNG

Die Schaukastenaudiometrie (Visual reinforcement audiometry) ist eine neue Methode welche für die Prüfung des Gehörs sehr kleiner Kinder gut geeignet ist. Diese Methode ist eine Verbesserung und Vereinfachung der von Suzuki und Ogiba ausgearbeiteten "conditioned orientation reflex audiometry". Zur Erreichung

## THE NYSTAGMUS THRESHOLD BASIS OF VESTIBULOMETRY

A. MONTANDON, S. HUGUENIN, A. ROHR, M. LUTET and M. PITTELOUD

*Clinique Oto-rhino-laryngologique de l'Université de Genève, Geneva, Switzerland*

New experimental research is presented on the determination of the parameters of stimulation and of measurement of the vestibular responses recorded by ENG with special mention of a procedure allowing the checking of the accuracy of the stimulating devices.

Because of its extreme complexity vestibular diagnosis requires very simple and accurate methods of investigation. Tests have to be always identical reproducible and measurable. They must permit also clear recognition of the limits between the normal and the pathological. A good example of clinical investigation of a sensory apparatus is tonal audiometry as it is practised nowadays, which can be used not only for qualitative, but also quantitative appraisals of its function.

The purpose of the Liminal Rotatory Test (LRT) we have earlier described (Montandon *et al.* 1954, 1956, 1961, 1965) is to define by means of a constant angular acceleration of  $1/s^2$ ,  $3/s$  and  $6/s$  from 0 to  $90/s$  of rotation speed, with an ENG record, at what level the nystagmic signal emerges. From that point it becomes possible to quantify the vestibular function in the sense of a vestibulometry comparable with audiometry. Therefore it is essential that the nystagmic threshold, which appears in form of an objective reaction, should be as exactly defined as the audiometric one which is a subjective sensation and which was defined by a statistical analysis.

### Definition

Despite the diversity of the criteria used by the authors—for example the sensation of rotation, the postrotatory nystagmus with extrapolation of a theoretical threshold, or the perrotatory nystagmus of angular acceleration—the value of the vestibular threshold has nevertheless been established around  $1/s^2$ . Mulder (1908)  $2/s^2$ , Buys (1910) about  $1/s$ , van Wulfften Palthe (1922)  $2/s^2$ , Arslan (1934)  $0.5-1/s^2$ , Groen & Jongkees (1948)  $0.5/s$ , Hulk & Jongkees (1948)  $2.5/s$  ( $0.75$  to  $5/s$ ), Hilding (1953)  $0.7-1/s$  ( $0.25-3/s^2$ ), Hood (1966)  $1-2/s$ , Kaphan (1968)  $1/s$ . Others, like de Vries & Schierbeek (1933) and Hoggeveen & Nijhoff (1956) have tried to obtain an instantaneous threshold using very short stimulation of

This work was supported by grant from the Swiss National Foundation for Scientific Research.

apparent improvement in hearing in the growing child has lead many observers to think a growing child has improving hearing. This sometimes leads to false conclusions. Our experience is that with surmounted evoked cortical potentials following sound stimuli, values nearer the true thresholds in children under 2 / years are obtained, than by the use of various type tests.

Finally I would like to ask Dr Lidén at what intensity values he would accept an audiogram as normal in the case of a 6-year-old child.

A *Burian* Wir haben eine Gruppe von 50 Kindern im Alter zwischen 6-12 Monaten mit der Methode der Baby Bett Audiometrie (nach Bisalski) untersucht. Dabei ergab sich eine Hörschwelle zwischen 30-40 dB. Wir haben die gleiche Gruppe von Kindern mit Hilfe der Evoked Response-Audiometrie einer Kontrolluntersuchung unterzogen. Dabei ergab sich eine objektive Hörschwelle von 20-25 dB. Diese objektive Hörschwelle fällt mit zunehmendem Alter ab und erreicht in Alter zwischen 4-6 Jahren eine Schwelle von 0-5 dB.

G *Lidén* (Reply) to Mr *Laskiewicz* The reason that I did not discuss the paper of Dix & Hallpike is that I did not want visual reinforcement audiometry to be confused with their method. In visual reinforcement audiometry the child is not instructed to respond in a particular way. Thus no active cooperation of the child is required as in the peep-show technique according to Dix & Hallpike.

To Mr *Bordley* At 6 years of age the tone thresholds are very close to that of adults.

TABLE 1 Vestibular nystagmus threshold of angular acceleratory nystagmus recorded on 139 normal human subjects

Author	Number of cases	Mean value (calculated)	95 % of the cases are comprised between the 1 $\sigma$ values
Flornbach, 1835	34	0.77 $\mu$ s	0.59-0.91 $\mu$ s
Lummeaux, 1938	105	0.92 $\mu$ s	0.76-1.08 $\mu$ s

Experimentally that couple may be demonstrated using the stereotaxic method on rabbits. By means of a continuous electric stimulation of the Diencephalic Nystagmogenic Area, at a frequency of 35 Hz, a nystagmic reaction is induced at a definite level of intensity (0.5-1  $\mu$ ). This diphasic nystagmus of central origin is absolutely similar and correlated to the nystagmus provoked by rotatory stimulation: threshold latency time of a few seconds, increase of the frequency and maximum frequency rate corresponding to the intensity of stimulation, and poststimulatory nystagmus evoking the postrotatory nystagmus (Monnier M & Montandon, P., 1962; Montandon, P. 1964; Montandon P & Monnier M. 1964; Montandon, A. *et al* 1963, 1965, 1967).

The same correlation must be realized in clinical work using a continuous angular acceleration by means of a turning system. In order to obtain a constant rotatory stimulation, reproducible and of a definite value allowing a clinical valuation of the nystagmic threshold, every turning device has to be carefully standardized and subsequently controlled.

In this connection it must be recalled that the inscriptions on the dial are usually not accurate, that the setting of the equipment is frequently out of order and that the tachometer connected directly on the motor does not give reliable information on what happens to the turntable itself. The only measurements which can be considered reliable are those made directly on the turntable.

During a two-year period of trial in our laboratory many testing procedures of the accuracy of the rotating devices have been checked, showing the considerable importance of this factor in clinical vestibulometry. Practically we suggest a simplified chronometric method which may be used by every operator (Table 2 and comments.)

TABLE 2

Angular acceleration (1 $\mu$ s)	Time in seconds for			
	$\frac{1}{2}$ turn	1 turn	1 $\frac{1}{2}$ turn	2 turns
Calculated time	19.0	26.8	32.9	37.9
Lower limit	18.4	26.1	32.0	36.9
Upper limit	19.6	27.6	33.8	39.0

0.4 s That procedure does not consider "the time element which is an important factor with a definite latent period of a few seconds at the low rates (Hilding 1953)

Our personal definition of the nystagmic threshold as it appears in the limits of the Liminal Rotatory Test must be completed as follows in order to avoid any confusion *The threshold of the vestibular nystagmus is a frequency threshold. It corresponds to the lowest grade of intensity of a continuous and constant stimulation which is able to provoke and to maintain until the end of the stimulating period a nystagmic reaction of a definite direction and of one beat per second (1 H)*

### Remarks

I This definition can also be applied to the central nystagmus issued from an electrical stereotaxic stimulation of the Diencephalic Nystagmogenic Area (DNA) as well as to the nystagmus provoked by an angular acceleration. The values in  $/s^2$  should be replaced by measurements in volts (0.75-1 v)

II The nystagmic reaction is a *rhythmic process* (Monnier 1967) of di-phasic beats, with a slow and a rapid phase which occurs in three stages: latency time, start of response and period of state (maximum frequency). *Only the last period of a steady maximum frequency must be taken into consideration to determine the threshold.* The two preceding periods, possibly reaching 10 to 20 seconds, are not suitable for a quantification. A minimum duration of 20 to 30 seconds of stimulation at a constant intensity is consequently required.

III Every reaction exceeding the period of stimulation corresponds to a supraliminal intensity level.

IV Other parameters, like amplitude or speed of the slow phase are not considered (frequency threshold).

According to the statistical analysis of two different series of researches carried out in our laboratory, the threshold of the angular acceleratory nystagmus recorded by ENG in complete darkness has been established at the values referred in Table 1.

It must be emphasized that

1 The level of  $1/s^2$  chosen as a *clinical threshold* is situated at the upper limit of the real threshold, which is usually lower.

2 Between the normal and the lowest pathological value at  $2/s^2$  the minimum gap reaches at least 100% which represents a very significant margin of security.

### Parameters of Stimulation

The existence of an accurate correlation, a so-called stimulation-response couple, is a necessary condition to define the threshold of the nystagmus.

TABLE 1 Vestibular nystagmus threshold of angular acceleratory nystagmus recorded on 139 normal human subjects

Author	Number of cases	Mean value (calculated)	95 of the cases are comprised between the two values
Rosbach, 1933	34	0.77 $^{\circ}/s^2$	0.50-0.91 $^{\circ}/s^2$
Permeaux, 1968	105	0.92 $^{\circ}/s^2$	0.78-1.09 $^{\circ}/s^2$

Experimentally that couple may be demonstrated using the stereotaxic method on rabbits. By means of a continuous electric stimulation of the Diencephalic Nystagmogenic Area, at a frequency of 35 Hz, a nystagmic reaction is induced at a definite level of intensity (0.5-1 v). This diphasic nystagmus of central origin is absolutely similar and correlated to the nystagmus provoked by rotatory stimulation *threshold* latency time of a few seconds, increase of the frequency and maximum frequency rate corresponding to the intensity of stimulation, and poststimulatory nystagmus evoking the postrotatory nystagmus (Monnier M & Montandon, P 1962 Montandon, P 1964 Montandon, P & Monnier M 1964 Montandon, A *et al.*, 1963 1965 1967)

The same correlation must be realized in clinical work using a continuous angular acceleration by means of a turning system. In order to obtain a constant rotatory stimulation, reproducible, and of a definite value allowing a clinical valuation of the nystagmic threshold, every turning device has to be carefully standardized and subsequently controlled.

In this connection it must be recalled that the inscriptions on the dial are usually not accurate that the setting of the equipment is frequently out of order and that the tachometer connected directly on the motor does not give reliable information on what happens to the turntable itself. The only measurements which can be considered reliable are those made directly on the turntable.

During a two-year period of trials in our laboratory many testing procedures of the accuracy of the rotating devices have been checked, showing the considerable importance of this factor in clinical vestibulometry. Practically we suggest a simplified chronometric method which may be used by every operator (Table 2 and comments.)

TABLE 2.

Angular acceleration ( $^{\circ}/s^2$ )	Time in seconds for			
	$\frac{1}{2}$ turn	1 turn	1 $\frac{1}{2}$ turn	2 turns
Calculated time	19.0	26.8	32.9	37.9
Lower limit	18.4	26.1	32.0	36.9
Upper limit	19.6	27.6	33.8	39.0



0.4 s That procedure does not consider "the time element which is an important factor with a definite latent period of a few seconds at the low rates (Hilding, 1953)

Our personal definition of the nystagmic threshold as it appears in the limits of the Liminal Rotatory Test must be completed as follows in order to avoid any confusion *The threshold of the vestibular nystagmus is a frequency threshold It corresponds to the lowest grade of intensity of a continuous and constant stimulation which is able to provoke and to maintain until the end of the stimulating period a nystagmic reaction of a definite direction and of one beat per second (1 Hz)*

### Remarks

I This definition can also be applied to the central nystagmus issued from an electrical stereotaxic stimulation of the Diencephalic Nystagmogenic Area (DNA) as well as to the nystagmus provoked by an angular acceleration The values in  $/s^2$  should be replaced by measurements in volts (0.75-1 v)

II The nystagmic reaction is a *rhythmic process* (Monnier 1967) of diphasic beats, with a slow and a rapid phase which occurs in three stages: latency time, start of response, and period of state (maximum frequency) *Only the last period of a steady maximum frequency must be taken into consideration to determine the threshold* The two preceding periods, possibly reaching 10 to 20 seconds, are not suitable for a quantification. A minimum duration of 20 to 30 seconds of stimulation at a constant intensity is consequently required

III Every reaction exceeding the period of stimulation corresponds to a supraliminal intensity level

IV Other parameters, like amplitude or speed of the slow phase, are not considered (frequency threshold)

According to the statistical analysis of two different series of researches carried out in our laboratory the threshold of the angular acceleratory nystagmus recorded by ENG in complete darkness has been established at the values referred in Table I

It must be emphasized that

1 The level of 1  $/s^2$  chosen as a *clinical threshold* is situated at the upper limit of the real threshold, which is usually lower

2 Between the normal and the lowest pathological value at 2  $/s^2$  the minimum gap reaches at least 100% which represents a very significant margin of security

### Parameters of Stimulation

The existence of an accurate correlation, a so-called "stimulation-response couple" is a necessary condition to define the threshold of the nystagmus.

Rabbit 1017 ES DNA right 0.5V

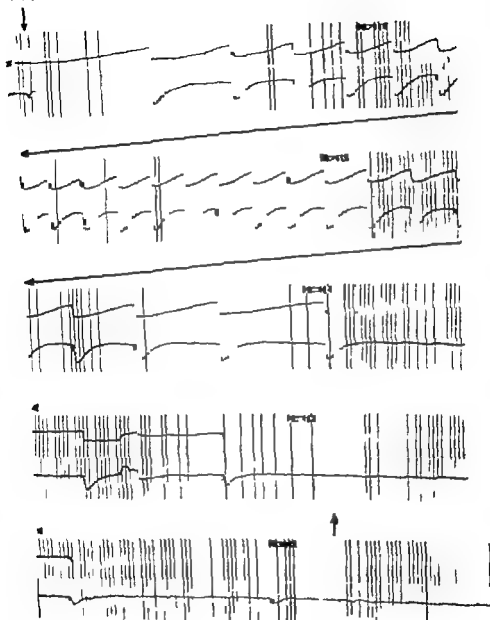


FIG. 2 Electric striaeotaxis stimulation of the right DNA just below threshold (0.5 Hz)  
 Direct coupling amplification (DC) ~ alternately coupling amplification (AC)

! Start of stimulation, † End of stimulation.

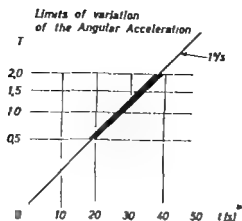


FIG. 1 Limits of allowed variation of the angular acceleration.

### *Simplified Chronometric Method to Check the Rotary Stimulating Device (for $1/s^2$ )*

1 Check constant speed ( $90/s$ ) and adjust if necessary. Measured time for 10 turns must be 40 seconds. Previously use acceleration of  $1/s^2$  approximately.

2 Check total acceleration time and adjust acceleration value ( $1/s^2$ ) if necessary. The time must be 90 seconds. (Points 1 and 2 not necessary if device functions correctly.)

3 Measure the time the turntable takes to make the first halfturn. Stop the device only after it has reached the speed of  $90/s$ .

4 Repeat point 3 for the first turn, turn and a half, double turn.

5 Repeat the whole test for different loads of the rotating chair.

The obtained values are compared with Table 2. The four points have to fall within the given limits. If it is not the case, the stimulating device cannot be considered as adequate to perform the Liminal Rotatory Test or to do any research on the response threshold.

### *Remarks*

The allowable errors in chronometry have been taken into account in the given limits.

Before starting the test, two marks have to be stuck on the turntable exactly  $180^\circ$  from each other.

This test takes into account the constance, the exact value ( $1/s^2$ ) and the reproductibility of the stimulation.

The lower and upper limits given are worst-case values (errors all adding up in the same direction). They correspond to an error of about 5% of the acceleration value ( $1/s^2$ ) and 0.1 s timing error. The measured time should be closer to the calculated theoretical time, as is the case for the practical measurements we performed on the Girograph in our laboratory (Fig. 1).

### Parameters of Observation

At the present time the nystagmus is most commonly recorded by the corneo-retinal potentials derived from periocular skin-electrodes (ENG). Our recording system includes two modes of amplification: the direct coupling (DC) which allows recording of the slow phase as well as the rapid phase, and the alternative coupling (AC) with a time constant of 0.3 s, which does not reproduce truly the eye movement but gives a perfectly stable ENG trace. Instead of DC, it should be sufficient to use only one AC amplifier with a longer time constant (e.g. 1.5 s).

The main parameter of the nystagmic threshold is the frequency: the nystagmus being a rhythmic process, therefore the defined threshold is a frequency-threshold (Figs. 2 and 3).

As for the amplitude of the beats, variations may occur depending on the procedure of recording, the type of apparatus, the filtration, and the placement of the electrodes. Therefore, this criterium is not valid.

The slow phase is often hardly recognizable if not accompanied by the fast phase of the nystagmus. According to our experience, the speed of the slow phase appears to be graphically correlated with the frequency if the amplitude remains constant. Its value is consequently submitted to the same uncertainty as amplitude. It is not a suitable criterium for the threshold.

As far as the choice of a method of recording is concerned it must be pointed out that every procedure using *visible light* even red light, *considerably disturbs the amplitude and the frequency of the nystagmus*. Therefore the recording has to be carried out in complete darkness.

### Clinical Applications

What is the practical meaning of the nystagmic threshold? It was experimentally demonstrated that each one of the DNA and of the peripheral labyrinth is normally responsible for one direction of the nystagmus, to the right or to the left. Each DNA is strictly unidirectional while each labyrinth is only partly unidirectional or at unilateral preponderance nearby the threshold. In order to differentiate the reaction of each labyrinth separately it is necessary to use very weak, precise, and constant acceleratory stimulations of long duration at the threshold value ( $1 \mu s^2$ ). From the nystagmic threshold it is then possible to evaluate the loss of function, the grade of central compensation, the masking intensity of a latent or spontaneous nystagmus, and to follow by an exact quantification the evolution of any vestibular dysfunction from central as well as from peripheral origin. Two years ago we published an ENG semiology based on 12,000 traces representing 2000 patients tested by this method of vestibulometry (Montandon, 1966).

## Rabbit 1017 ES DNA right, 0.75 V

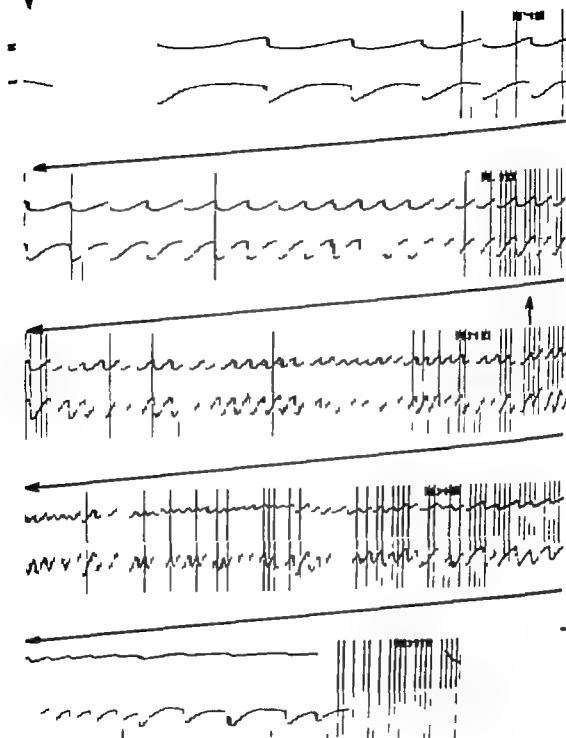


FIG. 3. Electrical stimulation of the right DNA below threshold (27 Hz) plus poststimulatory nystagmus. — Direct coupling amplification (DC) ~ alternating coupling amplification (AC).

↓ Start of stimulation. † End of stimulation.

- MONTAUDON, A. and MONTAUDON, P. 1963 Nystagme central et nystagme périphérique. *Act Otolaryng* (Stockh.) 50 320
- MONTAUDON, A. and R. REMACK, A. 1935 L'Épreuve giratoire Humaine. *Pract Otolaryng* (Basel) 27 224
- MONTAUDON, A., REMACK, A. and F. MEYER, J. 1936 Détermination du seuil vestibulaire par l'accélération giratoire, chez l'homme normal et en pathologie. *Compt. Rend. Acad. Sci. Paris* 301 212
- MONTAUDON, P. 1964 Functional correlations between the diencephalic vestibular area (DVA) and the vestibular nystagmogenic area (VNA). *Act Otolaryng* (Stockh.) 51 164
- MONTAUDON, P. and M. VERNER, M. 1964 Correlation of the diencephalic vestibular area with labyrinthine-vestibular nystagmogenic reflexes. *Brain*, 87 673
- M. LÖNN, W. 1908 Dissertation. Zürich (Cited by Roggvenen.)
- ROGGVENEN, B. L. and KUMAR, P. 1956 The normal and pathological threshold for the optokinetic illusion and the turning reaction. *Act Otolaryng* (Stockh.) 48 533
- ROMAN, A. 1907 Dissertation. Zürich (Cited by Roggvenen.)
- R. REMACK, A. 1966 Le seuil de nystagme d'accélération giratoire. Thesis. Geneva
- VERGÉ, H. L. DE and SCHUMACHER, P. 1953 The minimum perceptible angular velocity under motion condition. *Pract Otolaryng* (Basel) 35 60
- WILKINSON, P. and P. M. 1952 Function of the deeper sensibility and of the vestibular organs in flying. *Act Otolaryng* (Stockh.) 4 414

Prof. A. Montandon Clin. O.R.L.,  
Université de Genève  
Switzerland

## DISCUSSION

S. K. KINOSHITA: Mr. Montandon has recorded a central nystagmus induced by direct stimulation of the CNS and studied changes of this nystagmus under the influence of rotatory acceleration. Such studies may help to understand the mechanism involved in central nystagmus in man. But I am not sure that threshold studies will be a valuable in cases when the vestibular end-organ is impaired. In such cases the study of the slow component of the nystagmus under conditions of suprakinematic stimulation seems to be more important.

J. J. GROEN: Why does Mr. Montandon insist on the angular acceleration as the stimulus for determining the vestibular threshold? The acceleration takes long time to produce its maximum effect. Actually the product of acceleration and the duration of its application to produce the first nystagmic stroke (slow phase) is a constant, which is equal to the minimum angular velocity. This one constitutes the real vestibular threshold. So why not use an impulse as in optometry which produces the wanted value immediately?

G. F. GILL: La recherche pour préciser le seuil de la réponse nystagmique est de la plus grande importance et pour la réaliser il est évident que le stimulus doit être régulier et rigoureusement mesurable. C'est pourquoi nous utilisons la stimulation sinusoidale au cadre pendulaire. Ce moyen plus facile à réaliser permet de déterminer le seuil et donne ainsi en conséquence un renseignement très important.

L. B. R. J. NGKEE: Mr. Montandon defines the threshold for his experiments as the examination of the stimulus that gives a nystagmus till the end of the stimulation. This does not seem to me to be possible since during accelerating

## RÉSUMÉ

Nouvelles recherches expérimentales sur la détermination des paramètres de stimulation et de mensuration des réponses vestibulaires enregistrées à l'ENG, avec une mention spéciale d'un procédé permettant de vérifier le degré de précision de l'appareillage utilisé

## ZUSAMMENFASSUNG

Neue experimentelle Untersuchungen über die Bestimmung der Reizparameter und die Messung der registrierten vestibulären Antwort (ENG) mit spezieller Erwähnung einer Methode zur Kontrolle des Genauigkeitsgrades der verwendeten Einrichtungen

## REFERENCES

- ARSLAN, K. 1934. Nouvelles méthodes de sémiologie vestibulaire. *Rev. Laryng.* 55, 79.
- ASCHAN, E., NYLÉN, L. O., ST. HIL, J. and WERHILL, R. 1952. The rotation test. *Acta Otolaryng.* (Stockh.) 42, 431.
- BURN, E., 1910. Über die Nystagmographie beim Menschen. *Zbl. Ohrenheilk.* 9, 57.
- 1913. *Maschr. Ohrenheilk.*, 47, 575.
- FURBER, J. 1958. Détermination chez le sujet sain du seuil vestibulaire par une méthode clinique. Thesis. Geneva.
- GROSS, J. J. and JONAKHEA, L. B. W. 1948. The threshold of angular acceleration perception. *J. Physiol.* 107, 1.
- HILDI, G. A. C., 1953. Studies on the otic labyrinth. On the threshold of minimum perceptible angular acceleration. *Ann. Otol.* 62, 5.
- HOOD, J. D. 1960. *Myotatic kinesthetic and vestibular mechanisms*. Ciba Foundation Symposium, p. 245. London.
- HULL, J. and JONAKHEA, L. B. W. 1948. Turning test with small regulable stimuli. *J. Laryng.* 62, 63.
- КАРН, Ч. G. 1963. Personal communication.
- МОНЬЕР, М. 1967. *Central mechanisms of vestibular and optokinetic nystagmus*. Ciba Foundation Symposium, p. 205. London.
- МОНЬЕР, М. and MONTAUDON, A. 1962. Proc. XXII Congr. Physiol. Sci. Int. Excerpt. Méd. Int. Congr. Sci. 48, 1020.
- MONTAUDON, A. 1954. L'exploration des réactions vestibulaires par la méthode des stimuli giratoires différenciés (SGD). *Ann. Otol.* 31, 839.
- 1956. L'Épreuve Giratoire Liminaire dans les traumatismes crâniens. *C. R. l'Acad. Neurol.* 16, 197.
- 1961. Les critères physico-cliniques du seuil nystagmique vestibulaire. *Conf. Neurol.* 21, 447.
- 1963. Nystagmus giratoire et estibulométrie. *Arch. Suiss. Neurol. Psychiat. Psychiat.* 96, 268.
- 1966. Essai d'une sémiologie vestibulaire électro-nystagmographique (ENG). *Rev. Neurol. (Paris)* 117, 315.
- MONTAUDON, A., LAPORTE, J., Q. SCHAUD, F. and MONTAUDON, P. 1967. Les stimulations vestibulaires combinées. *Rev. Otol. neuro-ophthal.* 39, 283.
- MONTAUDON, A. and LIMON, J. 1963. Effet de l'appareillage expérimental sur l'ENG sur le nystagmus vestibulaire giratoire. *Acta Otolaryng.* (Stockh.) 56, 402.

## THREE DERIVATIONS IN ELECTRONYSTAGMOGRAPHY

M. PAVŠINI and I. PADOVAN

Zagreb, Yugoslavia

In order to be able to detect in the simplest possible manner and in part also to analyse the changed direction of nystagmus, three derivations for recording nystagmus have been introduced. Three bipolar leads are connected to three electrodes. The magnitude of the electric effect in one derivation is directly proportional to the corneoretinal potential in the second inversely proportional to the square of distance of the lead points from the rotating dipole both are corrected by calibration. The third magnitude of the electric effect is in direct proportion to the cosine of the deflection angle which exists between the rotating dipole and the line of derivation. It is thus possible to mark in an isocline for each derivation the amplitude of the deflection of the eye from the central point.

Electronystagmography has not, till now had a generally-accepted standardisation, only conventions. The active electrodes are situated at each outer canthus of the eye and the ground electrode is placed on the forehead in order to obtain the horizontal derivations for both eyes at the same time. In horizontal nystagmus the right-beating nystagmus is positive and lies above the zero line, while the left beating nystagmus is below the zero line.

The calibration is also conventional, having the patient alternate his gaze between two points representing a 10° arc of swing of the eyes so that the amplitude in the nystagmogram is 10 mm.

### *Three-channelled vectographic electronystagmography*

In order to see and analyse also the other derivations of the nystagmus besides the horizontal one we introduced three directions in the registration of the nystagmus. By doing so, we did not neglect the general desire for standardisation in nystagmography. We follow this form strictly so that we do not change either the number or the position of the electrodes or the raw or conventional channels.

The technique is as follows. The ground electrode situated on the forehead also becomes active, so that we have at the same time three channels instead of one, forming an isocline triangle. In this way every direction of the nystagmus can be situated and from the vectogram its direction and intensity determined.

From the three electrodes have three bipolar derivations

First channel electrodes 1-3

Second channel electrodes 1-2

Third channel electrodes 3-2



stimulation of constant magnitude the nystagmus always disappears after some 40 seconds.

*A Montandon (Reply)* We are grateful to our colleagues for their interesting discussions which show what important confusion still remains concerning vestibular threshold and especially the threshold of the nystagmus. Concerning our definition we must insist on two basic notions: the nystagmus is a rhythmic process and the threshold has been defined by the frequency of the ocular beats recorded in complete darkness by ENG. Experimentally the central permanent stereotaxic stimulation of the DNA gives rise to a quite similar reaction as the permanent application of a constant angular acceleration that is unidirectional nystagmus, with slow phase and quick phase frequency threshold corresponding to our preceding definition and progressive increase of intensity at supraliminal intensity levels of stimulation. Now we may add separately:

*To Mr Khechinashvili* We did not find any more information at constant amplitude of the nystagmus by considering the speed of the slow phase than only the frequency (as demonstrated by slides).

*To Mr Groen* The Mulder's law of the product of time  $\times$  intensity of stimulation is only valid during the setting up of the nystagmic reaction but not at the maximum frequency level.

*To Mr Greiner* I agree completely with the angular acceleratory threshold he has demonstrated in the pendular test.

*To Mr Jongkees* We cannot compare the postrotatory (or poststimulatory) nystagmus used in cupulometry with the perrotatory nystagmus. We are recording during the permanent stimulation whether central or peripheral, by angular acceleration in the Liminal Rotatory Test: the duration of the perrotatory nystagmus is normally of at least 80-90 seconds if you accelerate at  $1/s^2$  to constant speed of 90 degrees per second or 180 seconds if you accelerate till a speed of 180 degrees per second and of many minutes or even longer if using a central stimulation. There is practically no exhaustion of a nystagmus obtained by permanent stimulation contrary to the nystagmus succeeding to an instantaneous and strong shock (after discharge).

The quantity of the electric response in each channel is

- 1 Directly proportional to the corneoretinal potential
- 2 Indirectly proportional to the square of the distance between the issue points of the rotating dipole.
- 3 Directly proportional to the cosinus of the angle of derivation which is formed between the direction of the rotating dipole and the line of derivation and line of the channel.

### Calibration

The first channel gives the sum of the deviation of both eyes and is calibrated in the conventional way having the patient alternate his gaze between two points representing a 10° arc of swing of the eyes which is determined as the amplitude of 10 mm. To simplify the method, we did not calibrate separately the second and third channels but reduced during the horizontal calibration the amplitude in the second and third channels to 5 mm.

Because of the reduction of the electrical effect by the square of the distance, the second channel registers prevalently the deviation of the left eye and the third channel deviations of the right eye.

### Analysis of the three-channelled electronystagmogram

1 In horizontal deviations of the eye the amplitude in the second and third channels is equally large but of the opposite direction and their annulation proves that the eye moves only in the horizontal direction.

The horizontal nystagmus is seen in Fig. 5

2 In oblique nystagmus the amplitude in the second and third channels will not be equally large. The more the direction of the eye movement becomes vertical to the direction of the channel the smaller will be the amplitude in this channel.

In this way is registered an alternative fixation of two points under an angle of 10° but in an oblique line.

Fig. 2. Three electrodes represent three channels. Their connections and their position direction are indicated.

Fig. 3. Horizontal deviation of the eye to the right and its projections in the three channels.

Fig. 4. Calibration. The eye fixes alternately two points on a horizontal line.

Fig. 5. The left-beating nystagmus. Between the two thick lines is the time of 1 second.

Fig. 6. The oblique movement of the eye and the projection in three channels.

Fig. 7. Alternately fixation of two points. The eyes move from right and below to the left and upwards little above the horizontal line.

Fig. 8. Left-beating nystagmus. It is not pure horizontal nystagmus. According to the direction it corresponds to the movement of the eyes indicated in Fig. 7.

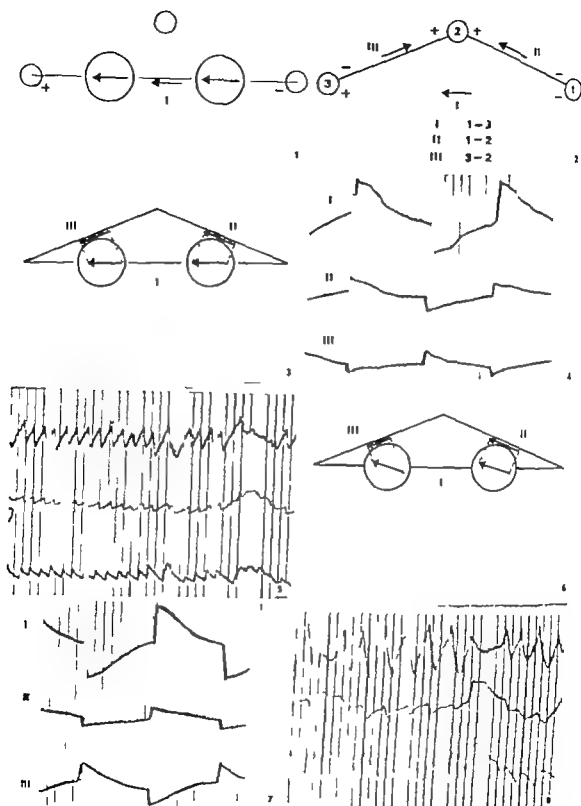


FIG. 1. Convulsive horizontal canal. The positive polarity is indicated at the right outer canthus of the eye, the negative at the left outer canthus, and the ground electrode at the forehead.

In a case where the direction of the deviation of the eye is perpendicular to the direction of a channel the pen movement in this channel is zero.

In the conventional electronystagmogram with one raw channel the analysis of the amplitude of the nystagmus and the velocity of the fast and slow components is exact only if the nystagmus is completely horizontal. The more oblique the nystagmus the bigger the error there does not exist a fixed system for finding the error and of correcting it.

In the three-channelled electronystagmogram it is possible to include a simple computer which is able to give, on the basis of the findings in the three channels, the exact direction of the nystagmus, the amplitude of the nystagmic movements, and the velocity values of the fast and slow components of every nystagmic movement.

3 In pure vertical nystagmus there is no response in the first channel while the second and third channels show the same amplitude and direction.

If the nystagmus is vertical but with a slight inclination to the right or left, there is a response also in the first channel which can, in the case of only raw derivation, lead to a false interpretation.

### CONCLUSION

Electronystagmography with three channel does not change the conditions of registration in the conventional raw derivation.

The ground electrode on the forehead is transformed to the active electrode so that three channels forming an isosceles triangle are obtained.

It is possible in this way to follow every direction of the nystagmus and to determine from the vectogram the exact amplitude and velocity of the slow component also in the case when the nystagmus is not horizontal.

By this method of registration of the nystagmus, errors in measuring the intensity of the nystagmus when it is not horizontal and the error of interpreting the vertical nystagmus as the horizontal can be avoided.

Three-channelled electronystagmography is applied in the registration of spontaneous nystagmus, of all sorts of positional nystagmus, and of induced nystagmus. It has become a routine electronystagmography.

FIG. 11 Left-beating nystagmus but with the direction perpendicular to the second channel.

FIG. 12 Left-beating nystagmus but with the direction perpendicular to the third channel.

FIG. 13 The movement of the eye in the vertical direction and the projection in the channels.

FIG. 14 The movement of the eye between the two points on vertical line.

FIG. 15 Vertical nystagmus which would not be registered in conventional raw derivation; in the first channel there is no pen movement.

FIG. 16 The vertical nystagmus upwards but with a slight inclination to the right. There appears too response in the first channel. In the second channel the amplitude is bigger than in the third one because it corresponds to the vectogram.

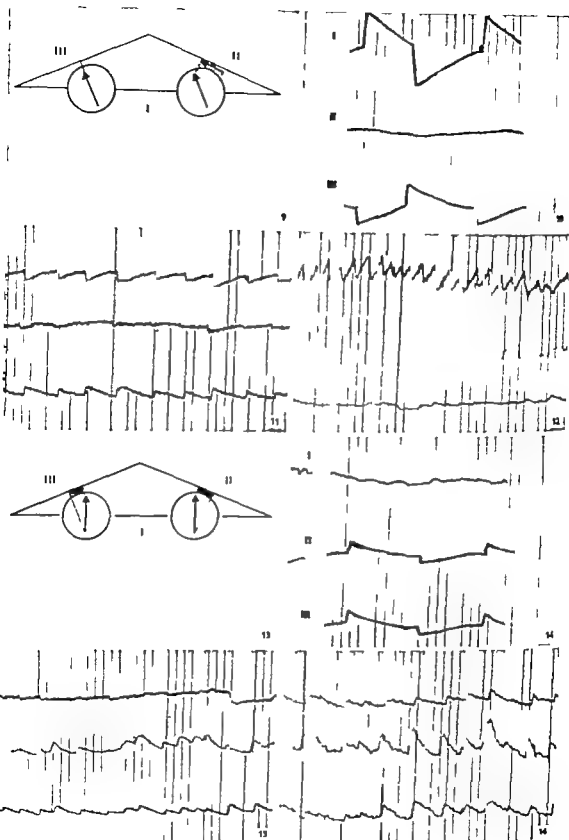


FIG. 9 The movement of the eye in the direction perpendicular to the third channel and the projection in the channels.

FIG. 10 Alternating fixation of the two points and the projection perpendicular to the second channel.

In a case where the direction of the deviation of the eye is perpendicular to the direction of a channel the pen movement in this channel is zero.

In the conventional electronystagmogram with one raw channel the analysis of the amplitude of the nystagmus and the velocity of the fast and slow components is exact only if the nystagmus is completely horizontal. The more oblique the nystagmus the bigger the error there does not exist a fixed system for finding the error and of correcting it.

In the three-channelled electronystagmogram it is possible to include a simple computer which is able to give, on the basis of the findings in the three channels, the exact direction of the nystagmus, the amplitude of the nystagmic movements, and the velocity values of the fast and slow components of every nystagmic movement.

3 In pure vertical nystagmus there is no response in the first channel while the second and third channels show the same amplitude and direction.

If the nystagmus is vertical but with a slight inclination to the right or left, there is a response also in the first channel which can, in the case of only raw derivation, lead to a false interpretation.

### CONCLUSION

Electronystagmography with three channels does not change the conditions of registration in the conventional raw derivation.

The ground electrode on the forehead is transformed to the active electrode so that three channels forming an isosceles triangle are obtained.

It is possible in this way to follow every direction of the nystagmus and to determine from the vectogram the exact amplitude and velocity of the slow component also in the case when the nystagmus is not horizontal.

By this method of registration of the nystagmus, errors in measuring the intensity of the nystagmus when it is not horizontal and the error of interpreting the vertical nystagmus as the horizontal can be avoided.

Three-channelled electronystagmography is applied in the registration of spontaneous nystagmus, of all sorts of positional nystagmus, and of induced nystagmus. It has become a routine electronystagmography.

FIG. 11. Left-beating nystagmus but with the direction perpendicular to the second channel.

FIG. 12. Left-beating nystagmus but with the direction perpendicular to the third channel.

FIG. 13. The movement of the eye in the critical direction and the projection in the channels.

FIG. 14. The movement of the eye between the 1 point on vertical line.

FIG. 15. Vertical nystagmus which would not be registered in conventional raw derivation; in the first channel there is no pen movement.

FIG. 16. The critical nystagmus upwards but with slight inclination to the right. There appears also response in the first channel. In the second channel the amplitude is bigger than in the third one because it corresponds to the vectogram.

In the dissociated nystagmus in which, instead of beating harmony of both eyes, only one eye will move while the other remains motionless, moves slightly or in the opposite direction three-channelled electronystagmography will warn us that we have to deal with a dissociated nystagmus because it will not be possible to compose the vectogram. In such a case registration by the other differentiated channels will be applied.

### RESUME

En vue de découvrir d'une manière simple et de pouvoir analyser partiellement la direction du nystagmus changée les auteurs ont introduit trois dérivation dans l'enregistrement du nystagmus. Au moyen de trois électrodes, les auteurs ont fait trois dérivation bipolaires. La dimension de l'effet électrique dans une dérivation est directement proportionnelle au potentiel corneo-rétinal la seconde est inversement proportionnelle au carré de la distance des points de conduite du dipol rotatif mais les auteurs les corrigent par la calibration. La troisième dimension de l'effet électrique est directement proportionnelle au cosinus de l'angle de la déclinaison existant entre la direction du dipol rotatif et la ligne de dérivation. De cette façon il est possible de tracer dans un triangle isocèle pour chaque dérivation l'amplitude de la déclinaison positive ou négative de l'oeil du point central.

### ZUSAMMENFASSUNG

Um die Richtungsveränderung des Nystagmus möglichst einfach zu beobachten und teilweise auch zu analysieren werden drei Kanäle in die Registrierung des Nystagmus eingeführt. Von 3 Elektroden werden die 3 bipolaren Derivationen entnommen. Die Grösse des elektrischen Effekts in einem Kanal ist direkt proportional dem corneo-retinalen Potential in einem zweiten indirekt proportional dem Quadrat der Entfernung der Derivationspunkte vom rotierenden Dipol, was durch Kalibrierung ausgeglichen wird. Im dritten ist die Grösse des elektrischen Effekts direkt proportional dem Cosinus des Deklinationswinkels der zwischen der Richtung des rotierenden Dipols und der Derivationslinie besteht. So ist es möglich, dass man für jeden Kanal in einem gleichschenkeligen Dreieck die Amplitude sowohl der positiven als auch der negativen Abweichung des Auges von dem Mittelpunkt eintragen kann.

### REFERENCES

- JONAS ZS, L. B. W. 1967 Vestibular tests. *Arch. Otolaryng. (Chic.)* 85 104  
 JONAS ZS, L. B. W. and PHILIPPOU A. J. 1964 Electronystagmography. *Acta Otolaryng. (Stockh.)* Suppl. 189  
 PHILIPPOU, A. J. 1967 Some aspects of electronystagmography. *J. Laryng.* 81 837  
 STANIL, J. 1955 Electronystagmography in the caloric and rotatory tests. *Acta Otolaryng. (Stockh.)* Suppl. 137  
 SMITH, C. R. 1967 Measurement of nystagmus using electronystagmography (ENG). *J. Speech Hearing Dis.* 32 133

J. Padovan M.D.  
 Gramscova Zagreb  
 Jugoslavia

## DISCUSSION

*U F Greiner* J pense qu la technique de Mr P dovan permet d mieux inscrire le ystagnus girat fro et de fl er les differents composants du mouvement du globe oculaire ce qui en fait n est pas réalisé par l'ENG habituell. J'aimerais savoir quelles sont ses électrodes et leur emplacement permettant les dérivation bipolaires.

*I Padovan (Réponse)* à Mr Greiner En ce qui co cerne les électrodes elles sont posées de la manière usuelle c'est à dire bitemporelle et sur le fro t. Le problème principal étant le troisième canal. Nous faisons nos recherches en collaboratio avec l'Institut de télécommunication.



## OBSERVATIONS UPON THE NERVOUS MECHANISM OF VESTIBULAR HABITUATION

M. R. Dix and J. D. Hood

*From the Medical Research Council National Hospital Queen Square London  
England*

Rotational tests carried out upon a number of ballet dancers have shown their nystagmic threshold responses to angular acceleration to be normal when determined in darkness, but considerably raised in the presence of optic fixation. These results indicate that optic fixation plays an important role in vestibular habituation in man and this, in turn calls for some reassessment of current views on its nervous mechanism.

Although, in contrast to caloric tests, rotational tests have limited localising value inasmuch as it is impossible by their means to examine separately the function of each labyrinth they do possess one singular advantage in that they enable precise and accurately graded stimuli to be applied at will. In this way the physical forces engendered within the canals may be determined and systematically related to the resultant nystagmic and other induced responses.

A feature of particular value in this respect is the ease with which rotational tests make possible the determination of the absolute sensitivity of the semi-circular canal mechanism. The information thus provided supplements that of the caloric tests, and has proved to be of considerable value in studies of vertigo and in particular of vestibular habituation which is the subject of this communication.

The cupula of the horizontal semi-circular canals, of course responds only to angular acceleratory stimuli. This calls for a rotating chair of high precision. That which we have used is servo-mechanically controlled and by means of it angular acceleration from 0.1 to 10  $\text{s}^{-2}$  with intermediate values may be applied at will. The cupular response to a sustained angular acceleration is a progressive increase in its angular deflection to a maximum value which is approached asymptotically and reached effectively after some 20 seconds. For this reason it has been our practice to apply angular accelerations for 20 seconds followed by prolonged periods of rotation at constant velocity. The resultant nystagmus is recorded electro-nystagmographically in total darkness. The procedure for the determination of canal sensitivity is exemplified in Fig. 1. This shows the nystagmic responses of a subject to four different angular accelerations each beginning, for convenience with the chair at rest. It will be seen that the lowest acceleration is sublim

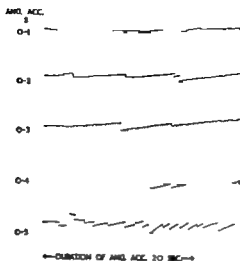


FIG. 1 Nystagmic response of normal subject recorded with eyes open in darkness to four angular acceleratory stimuli of 20 seconds duration.

inal and no nystagmus is detectable upon the recording. At  $0.2 \text{ s}^{-2}$  nystagmus begins to make its appearance and increases with further increase in angular acceleration.

The threshold response for this subject, therefore, lies somewhere between  $0.1$  and  $0.2 \text{ s}^{-2}$  and this figure provides a clear definitive measure of threshold sensitivity. The actual test procedures we adopt in practice is shown in Fig. 2. From rest, the chair is accelerated at  $0.5 \text{ s}^{-2}$  for 20 seconds, followed by a period of 60 seconds rotation at constant velocity. In the normal subject this induces a clear supraliminal nystagmic response. The chair is then accelerated at  $0.1 \text{ s}^{-2}$  for 20 seconds again, followed by 60 seconds rotation at constant velocity and this, in turn, is followed by a deceleration of  $0.1 \text{ s}^{-2}$  and so on with progressively increasing or decreasing values of angular acceleration or deceleration until a clear nystagmic response can be detected upon the recording. In this way not only can the threshold response be bracketed in much the same way as one determines the threshold of hearing, but this procedure also enables any directional preponderance present to be expressed in terms of threshold sensitivity in one direction or another. Normal threshold sensitivity appears to be of the order of  $0.15 \text{ s}^{-2}$ . This attests to the remarkable sensitivity of the cupular mechanism and indeed it seems not unlikely that in some individuals even higher sensitivities might well be revealed with further refinements in rotating chair design.

As a variant of this test procedure angular acceleratory thresholds may be determined in the same way with the subject fixating a light spot attached to and rotating with the chair. Optic fixation, of course, exerts a strong inhibitory effect upon induced nystagmus and, as a result, the stimu-

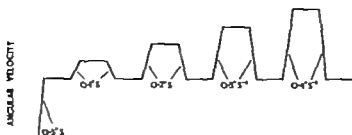


FIG. 2. Test procedure used for the determination of nystagmic threshold response to angular acceleratory stimuli

lus magnitude has on the average to be increased ten fold in the normal subject that is to say to  $1.5 \text{ s}^{-2}$  in order to evoke a just detectable threshold response. This factor of 10 we have referred to as the fixation index, since it would seem to provide a convenient expression of the magnitude of the restraint optic fixation exerts upon induced nystagmus, and it is with this that we shall be principally concerned in the foregoing. Let us now turn to the main topic of this communication namely so-called vestibular habituation.

As is well known repeated rotational stimulation about the vertical axis results in both man and animals in a progressive elimination of the reflex nystagmic eye movements which normally follow stimulation of the lateral semi-circular canals. The generally accepted view of the phenomenon is that it may be identified with habituation which Thorpe (1950) has defined as "an activity of the central nervous system, whereby innate responses to certain relatively simple stimuli, especially those of potential value as warning of danger wane as the stimuli continue for long periods without unfavourable results". In man the phenomenon finds its most common manifestation in ice skaters, ballet dancers and certain flying personnel who, in the course of their activities, are subjected to repeated rotational stimulation. As a result they appear to develop an immunity to the vertigo which would normally accompany excessive turning of the body in the inexperienced subject. In the past a number of studies have been carried out upon ice skaters and ballet dancers, the most notable being those of Mowrer (1934) and of McCabe (1966). Both authors comment upon the absence of nystagmus and vertigo in their subjects, either as a result of rotational stimulation or irrigation of the ears with ice water.

As a result of these and similar studies it has been widely assumed that the phenomenon has its origin in some central inhibitory mechanism which in some way not clearly understood, suppresses the normal physiological response of the vestibular end-organ.

Our own studies have been concerned with four ballet dancers, all of whom had had over seven years' experience of professional dancing.

Caloric tests were carried out on all four subjects according to the technique described by Fitzgerald & Hallpike and, in accordance with previous studies the responses were found to be either totally abolished or grossly reduced. This, as has been mentioned, has in the past been taken to imply bilateral abolition or reduction of canal sensitivity.

The results of the rotational tests, however carried out in the manner described, are shown in the following table and tell an entirely different story.

*Rotational Tests*  
*Average for normal subjects*

Angular acceleratory threshold of nystagmus in darkness	Angular acceleratory threshold of nystagmus with optic fixation	Fixation index
0.15 $\pm$	1.5 $\pm$	10

*Ballet dancers*

Subject			
1	0.1	8	80
2	0.2	6	25
3	0.2	10	50
4	0.2	8	40

Also are recapitulated the findings in normal subjects, namely angular acceleratory thresholds of nystagmus with and without optic fixation of 1.5 and 0.15  $\pm$  respectively giving a fixation index of 10. Below are shown the results, from the ballet dancers. The nystagmic thresholds in darkness, although marginally higher than the normal average, nevertheless seem to fall within the normal range, indicating in contrast to the caloric test results unimpaired semi-circular canal sensitivity. The thresholds obtained in the presence of optic fixation on the other hand are all considerably raised, giving fixation indices from 25 to 80.

It would seem, therefore, that what has happened in these subjects is not a suppression of canal sensitivity but the development of an abnormal capacity for maintaining visual fixation designed presumably to stabilise the eyes in the presence of disturbing vestibular activity. In other words a reinforcement of optic fixation.

Similar results to these have recently been reported by Collins (1966). Collins' investigations were carried out upon professional ice skaters. Amongst other studies he recorded the eye movements of the skaters, both with eyes open and closed, following piroettes. He found that, as was to

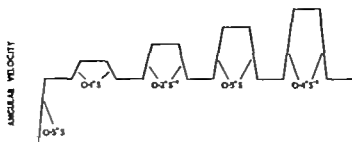


FIG. 2 Test procedure used for the determination of nystagmic threshold response to angular acceleratory stimuli.

lus magnitude has on the average to be increased ten fold in the normal subject, that is to say to  $1.5 \text{ s}^{-2}$  in order to evoke a just detectable threshold response. This factor of 10 we have referred to as the fixation index, since it would seem to provide a convenient expression of the magnitude of the restraint optic fixation exerts upon induced nystagmus, and it is with this that we shall be principally concerned in the foregoing. Let us now turn to the main topic of this communication, namely so-called vestibular habituation.

As is well known repeated rotational stimulation about the vertical axis results in both man and animals in a progressive elimination of the reflex nystagmic eye movements which normally follow stimulation of the lateral semi-circular canals. The generally accepted view of the phenomenon is that it may be identified with habituation which Thorpe (1950) has defined as "an activity of the central nervous system, whereby innate responses to certain relatively simple stimuli, especially those of potential value as warning of danger wane as the stimuli continue for long periods without unfavourable results". In man the phenomenon finds its most common manifestation in ice skaters, ballet dancers and certain flying personnel who in the course of their activities, are subjected to repeated rotational stimulation. As a result they appear to develop an immunity to the vertigo which would normally accompany excessive turning of the body in the inexperienced subject. In the past a number of studies have been carried out upon ice skaters and ballet dancers, the most notable being those of Mowrer (1934) and of McCabe (1960). Both authors comment upon the absence of nystagmus and vertigo in their subjects, either as a result of rotational stimulation or irrigation of the ears with ice water.

As a result of these and similar studies it has been widely assumed that the phenomenon has its origin in some central inhibitory mechanism which in some way not clearly understood suppresses the normal physiological response of the vestibular end-organ.

Our own studies have been concerned with four ballet dancers, all of whom had had over seven years' experience of professional dancing.

In any event our studies would appear to possess an immediate and practical application to clinical neuro-otology.

The finding of absence of conventional caloric responses with normal rotational thresholds and high fixation indices is by no means peculiar to ballet dancers. We have recently described elsewhere its occurrence in 78 of 274 patients with a diagnosis of vestibular neuronitis (Dix & Hood) in addition it is a not uncommon finding in subjects with extensive flying or sea-going experience.

Coles & Knight (1961) for example, noted diminished responses to conventional caloric tests in a group of experienced naval divers they attributed this to long-term habituation to the vestibular stimulation that occurs in diving operations from small craft. Later Coles (1968) has noted that seamen patients in general frequently give minimal or no response to conventional caloric stimulation, although when repeated with the patient's eyes open in the dark under electro-nystagmographic recording there is often a response within normal range of magnitude (as judged by maximum speed of the slow component of the induced nystagmus). He interprets the habituation he has observed in seamen as being mediated by enhancement of the inhibition produced by visual fixation.

Clearly therefore, bilateral abolition of the conventional caloric responses should never be taken to imply bilateral abolition or impairment of vestibular function. In all such cases the influence of optic fixation should be given special consideration. In this respect rotational tests carried out in the manner described can make a vital contribution to neuro-otological diagnosis.

## RÉSUMÉ

Des épreuves rotatoires avec des danseurs de ballet comme sujet ont montré, que leur se il ystagnique à l'accélération angulaire est normal, quand il est déterminé dans l'obscurité mais qu'il est considérablement élevé en présence d'une fixation optique. Ces résultats indiquent que la fixation optique joue un rôle important dans l'habituation vestibulaire et demandent pour cette raison une réexamination des vues courantes concernant le mécanisme nerveux de l'habituation vestibulaire.

## ZUSAMMENFASSUNG

Drehprüfung mit Ballettänzern zeigte einen normalen Schwellenwert für den Beschleunigungsnyctismus in Dunkelheit. Im Gegensatz dazu ist der Schwellenwert mit optischer Fixation erhöht. Diese Resultate zeigen dass die optische Fixation eine wichtige Rolle in der vestibulären Habituation spielt und machen damit eine Widererwägung der üblichen Ansichten über den nervösen Mechanismus der Habituation notwendig.

be expected from the results of earlier studies, nystagmus was strongly suppressed with eyes open by contrast however quite vigorous nystagmus appeared with eyes closed. Furthermore at the end of the pirouette his subjects had no difficulty in walking with eyes open, but experienced marked disorientation with their eyes closed. In addition, both caloric and rotational tests carried out upon his subjects with eyes open in darkness induced enhanced nystagmic responses not present with optic fixation. The close correspondence of Collins' results upon ice skaters and our own upon ballet dancers calls for special comment.

In pirouettes, ice skaters attain extremely high angular velocities of the order of 250-300 rpm and during this time the head is usually maintained in rigid conformity with the body. Ballet dancers, however as is well known, invariably fix their gaze to a particular object and maintain their heads in a fixed position in space while the body rotates. At each complete revolution the head is rapidly rotated and the object re-fixed. It is commonly believed that this manoeuvre eliminates the post-rotatory stimulus that would otherwise result and consequently accounts for the absence of vertigo. The theoretical considerations underlying this assumption cannot be questioned. On the other hand, if the practice conformed to the theory ballet dancers should not habituate since they would never experience unphysiological rotational stimuli. Since, as has been shown, habituation in these subjects only occurs in the presence of optic fixation a more likely explanation is that ballet dancers have developed a subconscious awareness of the fact that the suppression of their vertigo is in some way dependent upon optic fixation and use it accordingly to good effect.

This specificity of the phenomenon to the participation of optic fixation is in accord with the recent experimental observations of Marshall & Brown (1967). These authors showed that when normal individuals were subjected to repeated rotational tests in darkness there occurred a marked decline in the resultant nystagmic responses yet when the test stimulus was repeated with vision permitted no decline was apparent. In other words, the development of habituation in their subjects was specific to darkness. Our own observations clearly indicate that the same specificity and non-transference of habituation occurs in the presence of optic fixation.

As to the theoretical implications of these findings the phenomenon that we have observed is almost certainly central in origin and by analogy with recent pharmacological and electro-physiological experiments upon animals may possibly be associated, with certain presynaptic inhibitory mechanisms located in the pontine medullary portion of the reticular formation. Beyond this little more can be said at the moment. Whether the phenomenon we have observed can truly be described as habituation as defined by Thorpe is open to question. Collins (1968) as a result of his comparable studies upon ice skaters questions the use of this term and prefers to regard the phenomenon as a modification in form of the vestibular responses rather than a simple "dropping out".

In any event our studies would appear to possess an immediate and practical application to clinical neuro-otology.

The finding of absence of conventional caloric responses with normal rotational thresholds and high fixation indices is by no means peculiar to ballet dancers. We have recently described elsewhere its occurrence in 78 of 274 patients with a diagnosis of vestibular neuronitis (Dix & Hood). In addition it is a not uncommon finding in subjects with extensive flying or sea-going experience.

Coles & Knight (1961) for example, noted diminished responses to conventional caloric tests in a group of experienced naval divers. They attributed this to long-term habituation to the vestibular stimulation that occurs in diving operations from small craft. Later Coles (1968) has noted that seamen patients in general frequently give minimal or no response to conventional caloric stimulation, although when repeated with the patient's eyes open in the dark under electro-nystagmographic recording there is often a response within normal range of magnitude (as judged by maximum speed of the slow component of the induced nystagmus). He interprets the habituation he has observed in seamen as being mediated by enhancement of the inhibition produced by visual fixation.

Clearly therefore, bilateral abolition of the conventional caloric responses should never be taken to imply bilateral abolition or impairment of vestibular function. In all such cases the influence of optic fixation should be given special consideration. In this respect rotational tests carried out in the manner described can make a vital contribution to neuro-otological diagnosis.

## RESUME

Des épreuves rotatoires et des danseurs de ballet comme sujet ont montré, que leur seuil nystagmique à l'accélération angulaire est normal, quand il est déterminé dans l'obscurité, mais qu'il est considérablement élevé en présence d'une fixation optique. Ces résultats indiquent que la fixation optique joue un rôle important dans l'habituation vestibulaire et demandent pour cette raison une réexamination des vues courantes concernant le mécanisme nerveux de l'habituation vestibulaire.

## ZUSAMMENFASSUNG

Drehprüfung mit Ballettänzern zeigt, daß ein normaler Schwellenwert für den Beschleunigungsnystagmus im Dunkeln im Gegensatz dazu ist der Schwellenwert mit optischer Fixation erhöht. Diese Resultate zeigen, daß die optische Fixation eine wichtige Rolle in der vestibulären Habituation spielt und man hiermit in Widererwägung der üblichen Ansichten über den nervösen Mechanismus der Habituation notwendig.



## REFERENCES

- COLES, R. R. A. 1968 Personal communication
- COLES, R. R. A. and KNIGHT J. J. 1961: Aural and audiometric survey of qualified divers and submarine escape training tank instructors. *Med Res Comm Rep R.A.P.* 61 1011
- COLLINS, W. F. 1966 Vestibular responses from figure skaters. *Aerospace Med* 37 1098.
- 1968: Special effects of brief periods of visual fixation on nystagmus and sensations of turning. *Aero space Med* 39 257
- DIX, M. R. and HOOD, J. D. Vestibular habituation, its clinical significance and relationship to vestibular neuronitis. *Proc. Boarding Society Vestibular Symposium* (Uppsala 1968) In press.
- MCCABE, B. F. 1966 Vestibula suppression in figure skaters. *Trans Amer Acad Ophthal Otolaryng* 61 284
- MARSHALL, J. E., and BROWN J. H. 1967 Visual arousal interaction and specificity of nystagmus habituation. *Aerospace Med* 38 597
- MOWERS, O. H. 1934: The modification of vestibular nystagmus by means of repeated elicitation. *Comp Psychol Monog* 9 48
- THORPE, W. H. 1959 The concepts of learning and their relation to those of instinct. Physiological mechanisms in animal behaviour. *Symp. Soc Exp Biol* 4 337 Cambridge University Press

Medical Research Council  
National Hospital Queen's Square  
London W.C.1 England

## DISCUSSION

*A. Laskiewicz* Twenty five subjects submitted for repeated caloric and rotatory accelerating tests, 12 figure skaters, 10 ballet dancers, and three cases of post-traumatic head sequelae, have shown a clear response decline which was considerably influenced by optic fixation and was more significant with increased speed of turning or caloric stimuli. In three post-traumatic cases the habituation from vestibular responses, in which slow phase reached a distinct subnormal amplitude, was considerably weak marked. Postmortem examination of the brain in one of the named cases of traumatic injury of the occipital region has shown multilocular extravasations within the pontine part of the brainstem, including the reticular formation. This in particular seems to confirm the theoretical considerations on dependance of the cochleo-vestibular habituation from the normal function of the pontine segment of reticular formation.

*A. Montandon* In order to have a scale of comparison may I ask according to what definition of the vestibular nystagmic threshold your determination was done?

*C. F. Greiner* Je voudrais leur demander leur opinion sur l'habituation. En effet avec la stimulation sinusoïdale dans l'obscurité nous constatons qu'il n'y a pas d'habituation chez le sujet normal si bien que dans les expertises par ex nous considérons que l'apparition d'une habituation est en faveur d'une lésion des voies vestibulaires. C'est la raison pour laquelle nous exécutons 1) le vestibulaire 1 par stimulation angulaire sinusoïdale dans l'obscurité 2) par stimulation calorifique dans l'obscurité avec ENG 3) par stimulation, laécille avec nouve

ment des globes oculaires fixant un objet exécutant des mouvements pendulaires  
4 par stimulation avec rotation cervicale angulaire la tête étant maintenue im-  
mobile dans l'obscurité pour éliminer la fixation visuelle

L'interprétation de l'ensemble de ces investigations apporte des renseignements cliniques extrêmement utiles au diagnostic.

M Portmann La communication de Miss Dix et Mr Hood est très intéressante  
J voudrais rappeler l'importance des stimulations intenses répétitive pour la  
durée des séjours. L'un de nos collaborateurs Mr Beauchamp a (en 1950)  
# dit le *potogramme* (sensation et électroystagmographie) de pilotes de  
jet de l'armée d'air il a constaté après un certain nombre d'heures de vol  
l'inhibition considérable de la réponse subjective (sensation rotatoire) alors que  
la réponse ystagnique était presque la même. Ce type d'adaptation portant exclu-  
sivement sur la sensation et non sur le mécanisme vestibulaire lui-même est bien  
un phénomène purement central. Il doit être également pris en considération.

L. B. H. Jongkees Ballet dancers may fixate during their performances. Dance  
ing derwishes, however have an inward look i.e., they do not fixate. Neverthe-  
less, it shows perfect habituation.

On this point gives me problems. Mr Hood showed us that the caloric test,  
in subject with closed eyes, gives more information about the peripheral and  
in the subject with open eyes tells us more about the central vestibular system.  
How then can we use the Fitzgerald-Hallpike diagram (based on tests in patients  
with open eyes) to diagnose canal paresis?

S. Khechinashvili Mr Hood was very careful when he stated that he is not  
quite sure whether this phenomenon may be defined as habituation. I think that  
it is one of the various manifestations of the most complicated process of vesti-  
bular habituation and if we want to speak about its central mechanism, then in  
the first line central inhibition should be considered. During the last decades  
nystagmography has given us the opportunity to study an entirely new phenome-  
non—the nystagmic eye movements occurring in darkness and behind closed eye  
lids. This technique gives the opportunity to reveal also new aspects of habitua-  
tion and generally speaking of all compensatory phenomena going on in the  
vestibular system. Of course these two conditions, i.e. examination in darkness  
and with closed eyelids, are not identical because in the second case the involve-  
ment of sleep mechanism is more probable. It is of interest to know whether the  
finding in darkness and with closed eyelids were the same. If there was any  
difference then the possibility of activation of sleep or other central mechanisms  
should be considered.

C. F. Pfaltz I can confirm the hypothesis of Miss Dix and Mr Hood that opti-  
fixation is one of the most important factors involved in vestibular habituation.  
This central phenomenon can also be evoked by repeated caloric stimulations but  
only if the stimuli are applied sinusoidally and with optic fixation.

J. D. Hood (Reply) to M. Laskleson-Hernandez-Péon & Bruni Carmona have  
shown that in cat habituation is unaffected by lesions transecting the brain stem  
at the mid-collicular level, indicating the lower brain stem as the probable source  
of inhibitory mechanisms. How far however the results of these and similar ani-  
mal experiments can be applied to man is an open question, since there appear  
to be certain important differences between the development of the response de-  
fined in man and in animals.

To M. Montland: The method we have used to determine the threshold sensi-

## REFERENCES

- COLLA, R R A 1968 Personal communication
- COLLA, R R A and KNIGHT J J 1961 Aural and audiometric survey of qualified divers and submarine escape training tank instructors. *Med Res Councl Rep, R\J P* VI 1011
- COLLINS, W E., 1960: Vestibular responses from figure skaters. *Aerospace Med* 37 1094.
- 1968 Special effects of brief periods of visual fixation on nystagmus and sensations of turning. *Aerospace Med* 39 257
- DIX, M R., and HOOD, J D.: Vestibular habituation its clinical significance and relationship to vestibular neuronitis. *Proc Bårdny Society Vestibular Symposim* (Uppsala 1968) In press
- MCCABE, H F 1966 Vestibular suppression in figure skaters. *Trans Am r Acad. Ophthel Otolaryng* 64 254
- MARSHALL, J E., and BROWN, J H 1967: Visual arousal int reaction and specificity of nystagmus habituation. *Aerospace Med* 38 597
- MOWEN, O H 1934: The modification of vestibular nystagmus by means of repeated elicitation. *Comp Psychol Monog* 9 48
- THORPE, W H 1930 The concepts of learning and their relation to those of instinct. *Physiological mechanisms in animal behaviour Symp Soc Exp Biol* 4 387 Cambridge University Press.

Medical Research Council  
National Hospital Queen Square  
London WC1 England

## DISCUSSION

*A Laskienic* Twenty five subjects submitted for repeated caloric and rotatory accelerating tests, 12 figure skaters, 10 ballet dancers, and three cases of post traumatic head sequela, have shown a clear response decline which was considerably influenced by optic fixation and was more significant with increased speed of turning or caloric stimuli. In three post traumatic cases the habituation from vestibular responses, in which slow phase reached a distinct subnormal amplitude, was considerably weak-marked. Postmortem examination of the brain in one of the named cases of traumatic injury of the occipital region has shown multilocular extravasations within the pontine part of the brainstem, including the reticular formation. This in particular seems to confirm the theoretical considerations on dependance of the cochleo-vestibular habituation from the normal function of the pontine segment of reticular formation.

*A Montandon* In order to have a scale of comparison may I ask according to what definition of the vestibular nystagmic threshold your determination was done?

*G F Greiner* Je voudrais leur demander leur opinion sur l'habituatlon. En effet avec la stimulation sinusoidale dans l'obscurité nous constatons qu'il n'y a pas d'habituatlon chez le sujet normal et bien que dans les expertises par ex nous considerons que l'apparition d'une habituatlon est en faveur d'une lesion des voies vestibulaires. C'est la raison pour laquelle nous executons l'ex vestibulaire 1 par stimulation angulaire sinusoidale dans l'obscurité 2 par stimulation calorique dans l'obscurité avec ENG 3 par stimulation visuelle avec mouve-

# EVOKED RESPONSES IN THE INFERIOR COLLICULUS, MEDIAL GENICULATE BODY AND AUDITORY CORTEX BY SINGLE AND DOUBLE CLICKS IN CATS

## *A preliminary report*

B. ETHOLM

*From the Institute of Neurophysiology University of Oslo Oslo Norway*

This investigation shows that the auditory system has a high fidelity of the signal transmission. Using double clicks inhibitory processes were found lasting up to 130 msec and more in the medial geniculate body and the auditory cortex. In the inferior colliculus, inhibition lasted approximately 50 msec.

According to the travelling wave theory of Békésy (1947) a tone will activate a large part of the basilar membrane of the cochlea. The location of the area of maximum deflection depends upon the frequency of the tone. Correspondingly Katzung *et al* (1958) have found that single neurones in the lower region of the auditory system respond to tone bursts of a large frequency range. In the medial geniculate body (MG) however they found a much narrower frequency band to activate individual cells (Katzung *et al* 1959). They concluded that the analysis of the pitch of a complex sound is accomplished here. Because of the reduced active frequency range, it is likely that inhibition is involved in this analysing process.

It is the purpose of the present study to evaluate the inhibitory process at various stations of the auditory system and to see whether any inhibitory mechanisms in the medial geniculate body may explain the narrow frequency capable of driving individual MG cells.

## METHODS

Cats were anaesthetized with sodium pentobarbital (30 mg/kg). Clicks of short duration were delivered to one ear through one branch of a hollow Y-formed bar inserted into the external auditory meatus. The other branch was equally long and connected to a microphone. The microphone signals were amplified and displayed on an oscilloscope, giving the strength and timing of the click. After removal of the skull, the middle portions of the lateral supratylvian and ectosylvian gyri were removed by suction. After

tivity of the semi-circular canals is entirely empirical. It does, however possess the advantage of simplicity being dependent upon the detection of the absence or presence of a response, and in this respect is not dissimilar to conventional methods for the determination of the threshold of hearing, insofar as the liminal response may be bracketed between stimuli evoking a clear nystagmic response and stimuli evoking no response.

To Mr Greiner and Mr Pfaltz It is extremely interesting that habituation does not appear to occur from pendular stimulation. The reason for this is difficult to explain. It may be that the resultant constantly changing stimulus does not comprise the monotony which seems to be one of the necessary features for the development of habituation. Our own studies have been confined to subjects already habituated and we have carried out no systematic series of investigations upon normal subjects. The results of Marshall & Brown (1967) however would seem to suggest that habituation is specific to the stimulus conditions.

To Mr Portmann Mr Portmann's comments are extremely interesting and are in parallel with our own findings. They stress the need for careful enquiry into flying sea-going experience or any other occupation that might give rise to habituation in all patients found to have bilateral abolition or reduction of the caloric responses.

To Mr Jongkees The absence of vertigo in dancing derwishes may in part be explained by the fact that they rotate for very long periods at constant velocity. However as Mr Jongkees so rightly implies, movement of the head during this time must stimulate the vertical canals and one can only assume that these responses, too habituate though I am not aware of any studies specifically directed towards habituation resulting from stimulation of the vertical canals. With respect to canal paresis a bilateral paresis is a total abolition of the responses, as for example in streptomycin poisoning and in such cases there is, of course absence or reduction of the rotational responses both in the presence and absence of optic fixation.

Rotational tests, however have limited localising value, and it is difficult to see how they can provide any information upon unilateral canal paresis since both sets of canals are stimulated simultaneously.

To Mr Akchinskaya I do not think it is possible at this time to say with any certainty that the phenomenon we have studied is true habituation. In any event it does not seem to be vestibular suppression a term often used nowadays to describe the phenomenon in the sense that the end organ response is centrally inhibited. The question of sleep mechanisms complicating the interpretation of electro-nystagmographic recordings is extremely important. In this respect however recording in darkness with the eyes open and recording behind closed lids are not the same. Induced nystagmus recorded with eyes closed is much more likely to be influenced by environmental factors and by mental activity than when recordings are carried out with eyes open in darkness, and it seems not unreasonable to suppose that it is not the removal of vision but the act of closing the eyes which initiates sleep mechanisms, mediated I suppose by way of the reticular formation.

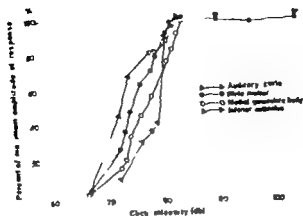


FIG. 2. Click-evoked responses in per cent of maximum amplitude of response from the inferior colliculus, the medial geniculate body, the white matter and the auditory cortex.

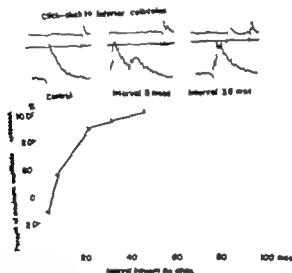


FIG. 3. The effect of conditioning click on the response to a test click recorded from the inferior colliculus. A variable interval between the two clicks. Both clicks had high intensity.

2 shows the relation between the strength of the click and the size of the responses obtained at the four stations, expressed as the percent of the maximum amplitude of the responses. The evoked potentials increased from 0 to 100 per cent over a range of click intensity of only 15 dB. The input-output curve was steepest for the responses of the colliculus inferior then followed the curves for the white matter and the medial geniculate responses, and at last, the auditory cortical responses.

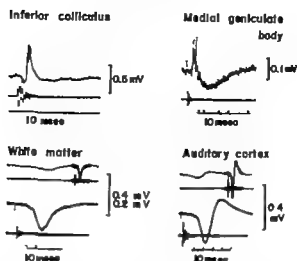


FIG. 1. Typical click-evoked responses recorded from the inferior colliculus, the medial geniculate body, the white matter and the auditory cortex.

exposure of the lateral ventricle, the fimbria and the hippocampus were removed with a glass seeker thus exposing the dorsal surface of the thalamus and the superior and inferior colliculi.

The recording electrodes were introduced through small holes in the pia on the dorsal surfaces of the thalamus and the inferior colliculus, respectively. Recordings from the inferior colliculus and the medial geniculate body were made with extracellular glass microelectrodes, whereas the white matter and cortical records were made with 0.2 mm thick platinum electrodes, insulated except at the tip.

## RESULTS

The first figure shows typical recordings from the inferior colliculus, the medial geniculate body, the auditory radiation (white matter) and the auditory cortex in response to a click of high intensity. In all records negativity is upwards. The second line in all records represents the microphone deflection signalling the intensity and the onset of the click at the eardrum. In the response from the colliculus inferior there was first a little positive wave, followed by a large negative wave with superimposed spikes representing the firing of the cells. The negative wave was usually followed by a positive wave although not distinct in this particular recording. In the recording from MG the sweep speed was higher. The response had almost the same configuration as in the inferior colliculus with an initial positive wave followed by a larger negative wave with spikes. However the late positive wave was more distinct and had a longer duration. In the white matter record, the response was a pure positive wave. With a surface electrode on the auditory cortex, the click evoked potential was the ordinary positive/negative biphasic primary evoked potential.

The auditory system shows a high fidelity of the signal transmission. Fig.

# RESUME

Les recherches présentées indiquent une transmission nerveuse de haute fidélité dans le système auditif. En utilisant des clics doubles, on a trouvé des mécanismes inhibiteurs durant jusqu'à 150 milli-secondes dans le corpus geniculatum médiale et le cortex auditif. Dans le colliculus inferior l'inhibition a duré environ 50 milli-secondes.

# ZUSAMMENFASSUNG

Die Untersuchung zeigt, dass Lautstimuli in dem auditorischen System leicht passieren. Durch Anwendung von doppelten Klicks hat man inhibitorische Phänomene gefunden die 150 Millisekunden oder mehr dauerten, sowohl in Corpus geniculatum mediale als im auditorischen Cortex. In Colliculus inferior dauerte die Inhibition ungefähr 50 Millisekunden.

# REFERENCES

1. DENNIS, P. ECKLES, J. C., and SAUND, T. A. 1964 The ventrobasal complex of the thalamus. Types of cells, their responses and their functional organization. *J. Physiol.* 174, 378.
2. BRIDLEY, G. VON, 1947 The variation of phase along the basilar membrane with sinusoidal vibrations. *J. Acoust. Soc. Am.* 19, 452.
3. RATER, L. Y. SAKA, T. UCHIYAMA, H., and W. ANGE, T. 1958 Electric responses of auditory neurons in cat to sound stimuli. *J. Neurophysiol.* 21, 549.
4. RATER, L. W. ANGE, T. and MURTY, V. 1959 Activity of auditory neurons in upper level of brain of cat. *J. Neurophysiol.* 22, 343.

Institut of Neur physiology  
University of Osl Oslo Norway

# DISCUSSION

A. Burian: Zu den sehr interessanten Ausführungen von Mr. Elholm möchte ich meine eigene Erfahrung aus der klinischen EEG-Computer-Audiometrie hinzufügen. Bei Patienten mit normalem EEG gelingt es mitunter nicht die akustisch evozierten Potentiale bei schwachen aber Stimulierung nachzuweisen. In solchen Fällen haben wir das Phänomen der Interaktion zweier akustischer Reize angewandt, das im Prinzip den Beobachtungen von H. Elholm entspricht. Dabei bietet man nach dem fraglichen, schwelennahen Prüfling mit einer Latenz von 120-150 msec einen zweiten sehr lauten Ton an, der sicher erkennbare Potentiale evoziert. Im Falle der erste schwelennaher Stimulus tatsächlich perzipiert wurde treten die beiden Potentiale in Interaktion. Dadurch wird die evozierte Antwort auf den zweiten Ton signifikante Weise verändert. Aus dieser Veränderung kann man auf die tatsächliche Perzeption des schwelennahen Reizeizes Rückschlüsse ziehen.

B. Elholm (Reph): Mr. Burian: Mr. Burian presented some very interesting results demonstrating that a stimulus with no measurable response still may have an inhibitory effect. I did not notice any time scale on the slide presented and wonder what the interval is between the two tones. In the future we have planned to do similar investigations.



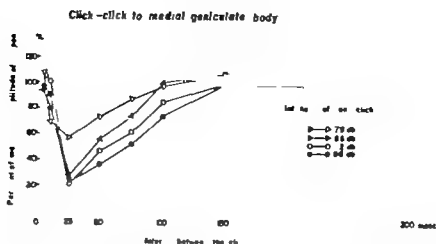


FIG. 7. Recordings from the medial geniculate body showing how the inhibition was dependent upon the intensity of the conditioning click.

culate body and further to the auditory cortex. All responses are normalized by setting the maximum response to 100 per cent. In the inferior colliculus, the inhibition was moderate. There was a great difference as the volley passed through the medial geniculate body. The added depression of the medial geniculate responses and of the recordings taken from the white matter indicates a marked inhibition of the medial geniculate neurones. In the white matter the inhibition lasted up to 150–200 msec. The conditioning click inhibited the response to the test click completely with an interval between the two clicks up to 100 msec. The recordings from the surface of the cortex were similar to the recordings from the white matter.

The inhibition is dependent upon the strength of the conditioning click. Fig. 6 shows responses from the inferior colliculus. Conditioning click intensities of 65 and 70 dB gave no inhibition. To the contrary, at an interval of 10 msec there was an increased response due to a summation of excitatory postsynaptic effects of the two clicks. With stronger shocks, inhibition occurred, the major part of the inhibition lasting only 25 msec.

In contrast to this behaviour Fig. 7 shows responses from the medial geniculate body. A conditioning click of an intensity of 79 dB produced a moderate inhibition. With increasing intensities of the conditioning click both the strength and duration of the inhibition were more pronounced.

The same phenomenon was seen when the recordings were made from the white matter. Here the inhibition was more pronounced than in the medial geniculate body. The auditory cortex showed similar responses.

The investigation has shown that there exists inhibitory mechanisms in the upper part of the auditory pathway. In the inferior colliculus, the inhibition is moderate and lasts only up to 50 msec. In the medial geniculate body the inhibition is stronger and has a longer duration. It is likely that the inhibition in the medial geniculate body is of both pre- and postsynaptic nature.

observers may need 60-90  $\mu$ sec. The instrument is connected to an audiometer via an interrupter which produces short tone bursts. The frequency of the tone can be selected on the audiometer. The interrupter unfortunately produces also a sharp click, being a more predominant cue for lateralization than the pure tone which follows. By using the click-free switch of the audiometer a transient free tone burst can be presented to the listener's ears. It appears that normal listeners are able to lateralize for time (-phase) differences with the aforementioned precision in the frequency range from low (e.g. 125 Hz) to about 800 Hz, beyond which frequency this ability rapidly declines. If the click is introduced lateralization is possible up to 4000 Hz although the precision is less than in the lower tone scale. The Medlophon guarantees the constancy of the intensities in the two headphones whatever time delay is introduced. This is extremely important for the following reason.

We have been using this instrument for several years on different kinds of patients, amongst whom were cases with disorders of the acoustic nerve, e.g. acoustic neurinoma. It appeared that in the latter group  $\Delta I$  discrimination was generally absent whereas interaural intensity differences  $\Delta I$  of normal magnitude (2 dB) already produced lateralization.

So, if we intend to investigate retrocochlear dysfunction, we should maintain a constant sound level but vary the interaural time delay  $\Delta t$  which is the most sensitive criterion for neural disorders.

In directional hearing both parameters phase (-time) difference  $\Delta t$  and intensity difference  $\Delta I$  play important roles. Even as low as 500 Hz a 2 dB intensity difference is attained already at an azimuth of 12° the higher the frequency the stronger the shadow effect of the head and the more the V cues will contribute to localisation. Nordlund (1963) worked with a loudspeaker in a free field, using pure tones in the range from 500-8000 Hz. Also bursts of noise (filtered) served as test sounds. We must assume that his patients had the benefit of both parameters. This may have been the reason that directional hearing was only impaired in cases of acoustic neurinoma but not abolished, because the interaural intensity differences  $\Delta I$  helped these patients to locate the sound source.

The ability to detect interaural time differences is highly developed in normal subjects as has been stated previously. It is worth while to go into this matter somewhat deeper.

If two identical sound signals are presented to the two ears almost simultaneously each cochlea will generate a volley group with a certain time pattern. This message travels via the acoustic nuclei to the accessory nucleus of the olive superior where it will meet its exact duplicate coming from the other cochlea. The conducting time from cochlea to olive is of the order of 3 milliseconds. Yet during this relatively long time, the time pattern of the volley group appears to be preserved with a precision better than 1% for 30  $\mu$ sec interaural time delay is already noticed and interpreted by the listener as just beside the midline. From this point of view it is plausible

## DIAGNOSTIC VALUE OF LATERALIZATION ABILITY FOR DICHOTIC TIME DIFFERENCES

J J GROEN

*From the Department of Labyrinthology Clinic for Ear Nose and Throat Surgery  
Utrecht the Netherlands*

The ability for lateralization of two sound signals, identical in all aspects except for time of arrival in the two ears, is an important diagnostic tool in the differential diagnosis between cochlear and retrocochlear deafness. A simple instrument for the lateralization test will be introduced and the results discussed.

The differential diagnosis between cochlear and retrocochlear dysfunction has gained considerably in reliability during the last 20 years. Nowadays a whole battery of audiometric tests helps us to localize the site of the lesion in the auditory pathway. Perceptive deafness of cochlear origin is relatively easy to diagnose. recruitment (and its allied phenomena) is the decisive symptom.

Less conclusive are the criteria for retrocochlear deafness. There is one exception. Different authors all agree upon type III of Jerger's classification: if the curves for interrupted and continuous tone presentation diverge strongly, a serious disturbance in the acoustic nerve is most probable. Slighter dysfunction of the acoustic nerve, however, may escape notice.

In the past several authors have recommended the use of directional hearing for the diagnosis of central auditory disturbances. In particular brain tumors in the temporal lobe would impair the precision in locating a sound source. Greene (1929) used a buzzing sound source in free field conditions and presented it also through a stethoscope. His results were that the ability to locate the source in free field or to lateralize it in stethoscopic presentation was significantly impaired in patients with tumors of the temporal lobe. Of the same opinion were Sanchez Longo & Forster (1957, 1958) who worked with the telephone receiver of a Maico audiometer: the latter switched to the position "masking" thus producing a harmonic complex consisting of multiples of 60 Hz.

Matzker & Welker (1959) described an instrument (Medlophon) which contains a variable delay line, enabling the investigator to introduce an adjustable interaural time difference  $\Delta t$  between otherwise identical signals.  $\Delta t$  can be regulated stepwise from 30  $\mu\text{sec}$  up to 648  $\mu\text{sec}$ . Normal listeners start to lateralize this dichotic sound presentation as soon as a time difference of about 30  $\mu\text{sec}$  is reached: none too careful but otherwise normal.

speech discrimination score was poor 20% at the best. Loss of coherence must have been caused here by too great a dispersion of conduction velocity in the neurons.

In all cases of perceptive deafness examined, we have encountered  $\Delta t$  discrimination inability only in VIII disturbances this is in agreement with the findings of Nordlund (1963) Unilateral disorders in the temporal lobe never abolish  $\Delta t$  perception, although the minimum  $\Delta t$  values may be increased slightly This is also in agreement with Nordlund's data, but is in contrast to the opinion of Sanchez Longo & Forster (1957)

The success of the  $\Delta t$  test has led to its frequent use in our audiology department—to such an extent that it would be worth while to look for a simpler form, easy to operate and manufacture. It should, however also fulfill the condition of constancy of sound level when interaural time difference is varied. A modified version of the stethoscope (see also Greene) was chosen (Groen, 1967) A rubber tube of 130 cm length, outer diameter 10 mm, inner diameter 7 mm, is connected to the tubes of a normal stethoscope frame. Over a distance of 15 cm to the left and right of the rubber center it is enclosed in a brass tube from which almost one half is removed, so that the rubber will slightly bulge over the edges of its brass housing. A frame with grip connects center and ends of this housing which also is engraved with cm markings to left and right of tube center The housing with grip is necessary in order to prevent the generation of highly disturbing contact noise the bare rubber tube certainly would produce even when handled with care (Fig. 2)

The test subject puts the earpieces of the stethoscope into his ears. The tube is very gently tapped by a leadpencil or similar implement at, e.g., 10 cm distance from tube center The test subject should be able to lateralize this presentation in the nearer ear If he does, the tube is tapped on the other side somewhat nearer to tube center Alternating left and right tapping and closing in on center the smallest distance from center is determined which just gives rise to a lateralization. If the ears of the patient are different a  $\Delta t$  hearing loss, midline localization will occur away from tube center towards the poorer ear This is explained by the time-intensity trade function of David *et al* (1958) a difference in loudness can be compensated for by a certain time-difference The poorer ear receives the message first and converts it into a neural signal. This will encounter longer synaptic delays than its stronger counterpart from the other ear and will arrive at the same time as this one in the olive The uncertainty in  $\Delta t$  around patient's center" should, however be the same as in normal listeners.

The tap on the tube wall starts off two travelling waves which have the same time pattern and sound spectrum, which, in our tube shows maxima at about 200, 400, and 600 Hz. Beyond 600 Hz the spectral envelope declines rapidly (Fig. 3) After about 2 msec the travelling waves will have changed into standing waves because of the finite length of the tube and the reflection gain at the closed endings. Difference in attenuation of the waves is

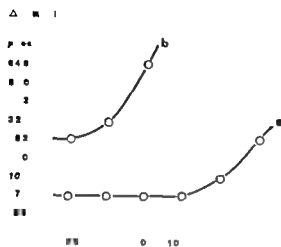


FIG. 1

FIG. 1 (a) Average Medlophon (with click)  $\Delta t$  data on a group of children with perceptive deafness of cochlear origin hearing loss between 40 and 85 dB in both ears (Fletcher average) Tube  $\Delta t$  data: 86  $\mu$ sec. (b) Average Medlophon (with click)  $\Delta t$  data on a group of children with perceptive deafness of retrocochlear origin (kernicterus) hearing loss between 35 and 85 dB (Fletcher average) Tube  $\Delta t$  data: from 400  $\mu$ sec to a measurable

FIG. 2. Tube  $\Delta t$  meter

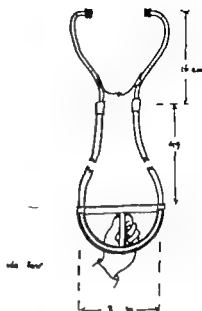


FIG. 2.

that even a minor disturbance in conducting properties of the nerve will endanger time pattern conservation and thus will reduce the ability for interaural time difference discrimination. This ability is not directly related to the pure tone audiogram (Nordlund). We found in a group of children (average age 12 years) with perinatal anoxia, having hearing losses ranging from 40 to 85 dB (Fletcher average) in both ears, that  $\Delta t$  values varied from 60 to 90  $\mu$ sec (thus in the normal range). In contrast to this, another group of children (average age also 12 years) with kernicterus at birth (due to Rhesus incompatibility of the parents) all having a sloping audiogram with a moderate loss of 45 dB (Fletcher average) yielded  $\Delta t$  values ranging from 400  $\mu$ sec to infinite (unmeasurable) (Fig. 1). A unilateral acoustic tumor even when pure tone hearing loss was moderate (e.g. 35 dB in the whole frequency range) led to complete abolition of interaural time difference discrimination. These patients keep hearing two separate sound impressions which will not fuse into a lateralized sound image. It appears that coherence in time pattern is lost when the signal is transported along the affected nerve rendering impossible the fusion with the signal coming from the other side into the olive. When asked how speech sounded through the affected ear as compared with the other one (normal) they said it sounded confused, echoic, as when spoken in a highly reverberating room. Their

speech discrimination score was poor 20% at the best. Loss of coherence must have been caused here by too great a dispersion of conduction velocity in the neurons.

In all cases of perceptive deafness examined, we have encountered  $\Delta t$  discrimination inability only in VIII disturbances. This is in agreement with the findings of Nordlund (1963). Unilateral disorders in the temporal lobe never abolish  $\Delta t$  perception, although the minimum  $\Delta t$  values may be increased slightly. This is also in agreement with Nordlund's data, but is in contrast to the opinion of Sanchez Longo & Forster (1957).

The success of the  $\Delta t$  test has led to its frequent use in our audiology department—to such an extent that it would be worth while to look for a simpler form, easy to operate and manufacture. It should however also fulfill the condition of constancy of sound level when internal time difference is varied. A modified version of the stethoscope (see also Greene) was chosen (Groen, 1967). A rubber tube of 130 cm length, outer diameter 10 mm, inner diameter 7 mm, is connected to the tubes of a normal stethoscope frame. Over a distance of 15 cm to the left and right of the rubber center it is enclosed in a brass tube from which almost one half is removed, so that the rubber will slightly bulge over the edges of its brass housing. A frame with grip connects center and ends of this housing which also is engraved with cm markings to left and right of tube center. The housing with grip is necessary in order to prevent the generation of highly disturbing contact noise the bare rubber tube certainly would produce even when handled with care (Fig. 2).

The test subject puts the earpieces of the stethoscope into his ears. The tube is very gently tapped by a leadpencil or similar implement at, e.g. 10 cm distance from tube center. The test subject should be able to lateralize this presentation in the nearer ear. If he does, the tube is tapped on the other side somewhat nearer to tube center. Alternating left and right tapping and closing in on center the smallest distance from center is determined which just gives rise to a lateralization. If the ears of the patient are different as to hearing loss, midline localization will occur away from tube center towards the poorer ear. This is explained by the time-intensity trade function of David *et al.* (1938): a difference in loudness can be compensated for by a certain time-difference. The poorer ear receives the message first and converts it into a neural signal. This will encounter longer synaptic delays than its stronger counterpart from the other ear and will arrive at the same time as this one in the olive. The uncertainty in  $\Delta t$  around patient's center should, however, be the same as in normal listeners.

The tap on the tube wall starts off two travelling waves which have the same time pattern and sound spectrum, which, in our tube shows maxima at about 200, 400 and 600 Hz. Beyond 600 Hz the spectral envelope declines rapidly (Fig. 3). After about 2 msec the travelling waves will have changed into standing waves because of the finite length of the tube and the reflection at the closed endings. Difference in attenuation of the waves is

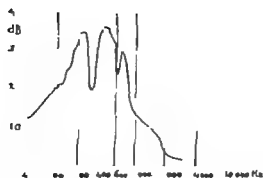


FIG. 3. Energy distribution as a function of frequency of a "tap" on the rubber tube.

negligible even for tapping at the extreme point near the end of the brass housing (15 cm from tube center) (Norton, 1935; Fay, 1940). If a bone conduction receiver is used in contact with the tube, lateralization occurs due to phase differences depending on the distance between contact point and tube center. This method has been used by van Soest & Groot (1929). It is a variation on the original work of Hornbostel & Wertheimer (1920). In the low frequency range the bone conduction receiver connected to an audiometer will give detailed information per frequency as to patient's ability to detect phase differences. As has been stated previously the tap produces a complex sound; the reactions of the patient then will pertain to his potentialities in the whole lower frequency range (up to 600 Hz) which is good enough for clinical purposes.

The margin of error of the normal listener is about 1 cm on the tube tapping should then take place at 0.5 cm from tube center (the distance to the nearer ear is  $2 \times 0.5 = 1.0$  cm shorter than to the farther ear). As the velocity of sound in the tube is practically 340 m/sec, the corresponding time difference then will be about 30  $\mu$ sec, which is the minimum delay normal listeners can detect. The average untrained listener will need some more, so 1.0 or 1.5 cm around tube center should be considered as a normal margin of error. 3 cm is already pathological.

In this way the  $\Delta t$  meter provides us with an easy method to detect abnormalities in 1st and/or 2nd order neurons. The instrument is easy to operate; the whole test takes about 1 minute; even a child can undergo it. Tube and Mediophon data obtained on different kinds of patients, are consistent. Whenever a patient fails to lateralize even with the maximum Mediophon delay (648  $\mu$ sec) he will not lateralize either with the tube and vice versa. Also the exact Mediophon delay times can be reproduced with the tube in terms of centimeters distance around the patient's center at the tube. Purely cochlear lesions do not disturb  $\Delta t$ -detection; middle ear abnormalities show normal  $\Delta t$  values, be it around a new midpoint shifted away from tube center in the direction of the worse ear.

## RESUME

Le pouvoir de latéraliser des signaux binauraux identiques en tous aspects, excepté pour le moment d'arrivée dans les deux oreilles, nous offre une méthode de diagnostic différentiel important entre surdité de perception cochléaire et rétrocochléaire. Un instrument simple pour ce test de latéralisation sera introduit et les résultats seront discutés.

## ZUSAMMENFASSUNG

Das Lateralisierungsvermögen für binaurale Signale, identisch in allen Hinsichten, nur verschieden in den Momenten der Ankunft in beiden Ohren ist ein wertvolles Messverfahren für die Differentialdiagnose zwischen cochleärer und retrocochleärer Schwerhörigkeit. Ein einfaches Instrument für die Lateralisationsuntersuchung wird introduziert und die Ergebnisse diskutiert.

## REFERENCES

- DIM, E. E., GUTMAN, Y. and BENNETT, W. A., 1936 On the mechanism of binaural fusion. *J.A.S.A.* 29 801.  
 F. R. D. 1940 Attenuation of sound in tubes. *J.A.S.A.* 12 62.  
 GROSZ, T. C., 1929 The ability to localize sound. A study of binaural hearing in patients with lesions of the brain. *Arch. Surg. (Chic.)* 18, 1625.  
 GROEN, J. J. 1947 *At-meting met twee. Ved. K.V.O. Heel.* in press.  
 HORNBOETEL, E. M. von, and WESTHEIMER, M. 1920: Über die Wahrnehmung der Schallrichtung. *Sitz. Ber. Akad. Wiss. (Berlin)* 15, 285.  
 MATEER, J. and WELSH, H., 1939 Di. Prüfung des Richtungshörs zum Nachweis und zur topischen Diagnostik von Hirnerkrankungen. *Z. Laryng. Rhin. u. Hals. u. Ohrenheilk.* 59 277.  
 NORDLUND, B., 1943 *Studies of otic cephalic hearing* Thesis, Göteborg.  
 ROSEN, H. A. 1935 Velocity of sound in small tubes. *J.A.S.A.*, 7 16.  
 SAVOUREY LOWE, L. P. and FORSTER, F. M. 1917 Clinical significance of impairment of sound localization. *Otolaryngology* 7 635.  
 — 1924 *Otolaryngology* 8 119.  
 SOROT, J. L. and GROOT E. D. 1929 *Physics*, 9 11.  
 — 1931 *Physics*, 11 103.

Dept of Otolaryngology  
 Clinic for Ear Nose and Throat Surgery  
 Utrecht the Netherlands

## DISCUSSION

© Lidé There has been much confusion concerning the diagnostic value of sound localization tests. Therefore it was very interesting to hear that Mr Groen has been able to confirm the result that Mr Nordlund in our laboratory has earlier presented with a free field sound localization test. As Mr Groen, we have found the sound localization test to be very sensitive for detecting early lesions in the eighth nerve.

J. E. Hordley This is a most important paper which Mr Groen has presented. It is his usual excellent manner. His observation concerning the possibility of the



delayed conduction time of the eighth nerve underlying this difficulty in localizing the direction of sound has been confirmed in another way by Shulzer in our laboratory. Timing the appearance of cortical potentials evoked by a sound stimulus, he has found an increased conduction time in the case of eighth nerve tumours. He has also found a shortened conduction time in cases of Menière's disease. We have not tested these cases for sound localization, but our findings would suggest that the shortened conduction might also result in difficulty in tone localization. If this is true we might have two different lesions causing localizing difficulty.

*J. J. Groen (Reply) to Mr Lidén* I am glad to learn that he is still of the same opinion as I am.

To Mr Bordley I want to thank Mr Bordley for his comments, especially on the work from his laboratory where EEG latency problems were studied in cases with peripheral tumors and in cases of Menière.

Whereas we find normal  $\Delta t$  values in Menière's patients, he finds shorter latencies than in normals. This discrepancy may perhaps be attributed to the difference in level on which these phenomena occur. In EEG audiometry these are cortical and in  $\Delta t$ -measurements they are on the level of the olivary complex.

## EEG-COMPUTER-ANALYSE — SINNVOLLE UND SINNVOLLE AKUSTISCHE REIZE

K. BURIAN, G. F. GESTRINO und H. HAIDER

*Amade Filialstation der Universitäts-IVVO-Klinik Wien Österreich*

Bei normalhörenden Versuchspersonen wurde eine vom Prüfling unabhängige Objektivierung des akustischen Diskriminationsvermögens angestrebt. Bei wiederholter Anbietung sinnloser oder sinnvoller Worte mit nachfolgendem Lichtreiz konnte eine Erwartungsstelle im Computer-gemittelten EEG abgewiesen werden. Dabei stellt das dem Licht blitz vorausgehende Wort einen Warnreiz dar, der die Ausbildung einer Erwartungsstelle vor dem Lichtreiz signalisiert. Solche Erwartungsstellen können selektiv durch die Kombination sinnloser oder sinnvoller Worte mit einem Lichtreiz ausgelöst werden. Nachdem eine derartige Selektion nur möglich ist, wenn die Versuchsperson den Bedeutungsgehalt der Testwörter differenzialisiert, ergibt sich daraus ein objektiver Hinweis auf deren Diskriminationsvermögen.

Die EEG-Computer-Audiometrie (ECA) vermittelt objektive Hinweise auf die akustische Perzeption, Reizleitung und Reizausbreitung im Cortex. Der Nachweis akustisch evokedter Potentiale sagt jedoch nichts über die grobstrukturelle Verarbeitung und Diskrimination der akustischen Signale aus. So kann es beispielsweise vorkommen, dass man bei Kindern, die als hochgradig schwerhörig gelten und auch typische Symptome dafür aufweisen, im ECA eine normale Tonhörschwelle registriert. Es kann sich dabei um eine Aphasie oder psychische Hörstörung handeln, die mit der bisher üblichen Technik der Click oder Sinustonsstimulierung nicht objektiviert werden kann. Ebenso wie in der subjektiven Audiometrie erst die Anwendung der Sprachaudiometrie die Beurteilung des Gesamthörvermögens ermöglicht hat, waren auch für die EEG-Computer Audiometrie Methoden erforderlich, die eine objektive und von der Mitarbeit des Patienten unabhängige Beurteilung des Sprachverständnisses und der Diskrimination gestatten. Einen ersten Beitrag zu diesem Problem stellen die folgenden Versuche dar.

Trotz der sehr umfangreichen Literatur über sensorisch evokierte Potentiale gibt es relativ wenig Arbeiten, die sich mit dem Problem der Diskriminierung sensorischer Reize befassen. Oswald *et al.* (1960) konnten bei Versuchspersonen während bestimmter Schlafstadien nach Anbietung verschiedener Wörter wesentlich häufiger K-Komplexe im EEG beobachten als nach akustischen Äquivalenten (Vor- oder Rückwärtsprechen be-



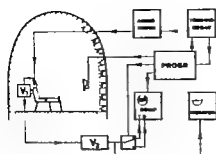


Abb. 1

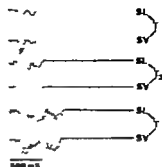


Abb. 2

Abb. 1 Block Schaltbild der gesamten Anlage. V Vorverstärker; V Hauptverstärker

Abb. 2. Summation der über Mikrophon aufgenommenen Wortreize der drei Testprogramme.

kommen einer Erwartungswelle ermöglicht werden. Schließlich wurde noch der Einfluss der Lautstärke auf das Diskriminationsvermögen untersucht, dazu wurden die Testworte vergleichsweise überaus hell (75 dB) und schwelennahe (25 bis 35 dB) angeboten. Die schwelennahe Intensität wurde individuell festgelegt und zwar derart, dass der Prüfling gerade noch alle Worte richtig nachsprechen konnte.

Die Ableitungselektroden wurden mit Hilfe von Bentonit Leitpaste auf den Vertex und auf das Mastoid (Referenz) aufgeklebt. Die abgeleiteten Potentiale wurden über Vor- und Hauptverstärker einer Schaltungsanlage zugeführt, welche die erhaltenen Informationen in zwei Kanäle eines Computers (CAT 400) aufteilte. Kanal 1 (in den Kurvenbildern oben dargestellt) analysierte das EEG während der sinnlosen und Kanal 2 (in den Kurvenbildern unten dargestellt) während der sinnvollen Wortstimulierung. Nach dem bei dieser Anordnung dieselben Elektroden und die gleichen Verstärker für die Analyse beider Wortreize verwendet werden, ist eine verlässliche Vergleichssituation gegeben. Die Testworte wurden zufallsverteilt auf eine Spur des Tonbandes aufgesprochen, auf der zweiten Spur des Bandes wurden Schaltimpulse aufgezeichnet, die den Beginn der Worte, getrennt nach sinn- und sinnlos, einem Programmierer zuführten, welcher die Synchronisation der Computeranalyse und der Kanalwahl vornahm (Abb. 1). Die Versuchsperson befand sich in einer schall- und echoarmen Kammer. Die akustischen Reize wurden durch Kopfhörer vermittelt, während in einem Blitzgerät zu gegebener Zeit die optischen Zweitreize produzierte.

Zur Kontrolle der gesamten Apparatur wurde das Programm anstelle der Versuchsperson über ein Mikrophon aufgenommen und gemittelt. Abb. 2 zeigt die Summation der sinnlosen (SL) und sinnvollen (SV) akustischen Reize der drei Testprogramme. Die Analysedauer betrug 2 Sekunden, die Zeitkonstante 1,2 Sek.

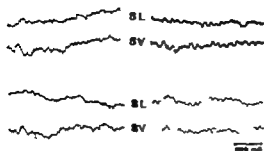


Abb. 3

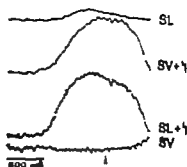


Abb. 4

Abb. 3 Vergleich der gemittelten Potentiale des Wortes "Fama" (obere Kurve) und "Mama" (untere Kurve) bei vier verschiedenen Versuchspersonen

Abb. 4 Typische Erwartungswellen, ausgelöst durch die Kombination eines sinnvollen und eines sinnlosen Wortes mit einem Lichtblitz (SL, Sinnloser Wortreiz SV, Sinnvoller Wortreiz)

## ERGEBNISSE

Bei dem ersten Test mit Unterscheidung zwischen nur zwei Worten zeigte sich, dass die evozierten Potentiale nach Anbietung des sinnvollen Wortes grösser und ausgesprägter waren als nach dem sinnlosen phonetischen Äquivalent. Der Grössenunterschied wies zwar eine beträchtliche Streubreite von wenigen bis über 100% auf, konnte jedoch immer erkannt werden (Abb. 3).

Mit einem zusätzlichen Lichtreiz nach jedem sinnvollen Wort konnte eine langsame negative Potentialschwankung im Sinne einer Erwartungswelle ausgelöst werden. Sie trat bei den meisten Fällen deutlich in Erscheinung, bei einigen wenigen Versuchspersonen waren sie nur schwach ausgebildet, aber erkennbar. Diese Erwartungswelle tritt zwischen dem Wort und Lichtreiz auf. Bietet man den Lichtreiz nach dem sinnlosen phonetischen Äquivalent an, dann kann man gleichfalls eine Erwartungswelle beobachten, wobei in dieser Versuchsanordnung das allein angebotene sinnvolle Wort (untere Kurve) als Vergleichsreiz dient (Abb. 4).

In einigen Versuchen liessen wir nach dem Lichtblitz eine motorische Reaktion ausführen (Handheben). In einer solchen Differenzierungssituation verstärkt diese die Erwartungswelle nicht oder nur ganz unwesentlich (Abb. 5). Unterschiede im Kurvenverlauf betreffen lediglich den Zeitpunkt der motorischen Reaktion selbst, nicht jedoch die Erwartungswelle. In den folgenden Versuchen haben wir daher diese Kombination nicht mehr angewandt.

Bei wiederholter Anbietung von nur einem sinnvollen und einem sinnlosen Wort wäre es möglich, dass die Versuchsperson durch die Wiederholung desselben sinnlosen Wortes dieses als gleichbleibendes Phonem in Erinnerung behält und somit zwischen der phonetischen Charakteristik, nicht aber zwischen dem Bedeutungsgehalt beider Worte differenziert. Wir

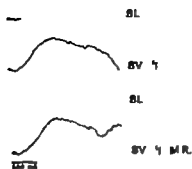


Abb. 5.

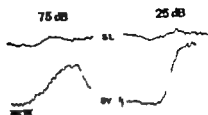


Abb. 6.

Abb. 5 Erwartungswelle bei Kombination eines sinnvollen Wortes mit einem Lichtblitz (oberes Kurvenpaar) und zusätzliche motorische Reaktion nach dem Lichtblitz (unteres Kurvenpaar)

Abb. 6 Erwartungswelle nach sinnvollem Wort und Lichtblitz bei 75 dB (oberes Kurvenpaar) und 25 dB (unteres Kurvenpaar)

haben daher in dem zweiten Versuch acht verschiedene sinnvolle Worte von acht unverständlichen phonetischen Äquivalenten unterscheiden lassen. Bei diesem Test zeigt sich der Unterschied im evokierten Potential des sinnlosen und sinnvollen Wortes nicht mehr so regelmässig und deutlich als im ersten Versuch. Bei der Kombination von sinnvollem Wort und Lichtblitz konnte jedoch wieder eine deutliche Erwartungswelle vor dem Lichtblitz nachgewiesen werden. Wurde zur Kontrolle der Lichtblitz nach dem sinnlosen Wort angeboten, so konnte nun die Erwartungswelle, ebenso wie bei dem vorangegangenen Versuch, auch durch das sinnlose Wort ausgelöst werden. Dieses Verhalten zeigt, dass die Versuchsperson den Warncharakter des Wortreizes im Hinblick auf den Lichtblitz als nachfolgendes Signal jeweils erkannt und somit zwischen sinnvollem und sinnlosem Wort unterschieden hat.

In dem dritten Versuch wurde die Differenzierungsleistung noch weiter gesteigert. Dabei wurden 30 verschiedene sinnvolle Worte und 30 sinnlose Buchstabenkombinationen zufallsverteilt angeboten, damit kommt es während des gesamten Testverlaufes zu keiner Wortwiederholung und die Versuchsperson muss den Bedeutungsgehalt jedes einzelnen Testwortes erkennen. Auch unter dieser Versuchsanordnung konnte eine typische Erwartungswelle nachgewiesen werden, wobei diese bei wechselnder Kombination des sinnvollen oder sinnlosen Wortes mit einem nachfolgenden Lichtblitz immer dort auftritt, wo der Lichtreiz angeboten wurde.

Bei Testung mit schwellennaher Intensität beobachtet man die gleichen Ergebnisse, wobei die Amplitude der Erwartungswelle oft noch grösser ist als bei überschwelliger Stimulierung (Abb. 6). Bedenkt man, dass die Versuchsperson bei schwellennahem Reiz eine wesentlich grössere Konzentration benötigt, um den Bedeutungsgehalt des angebotenen Reizes zu erfassen, dann bestätigt dies die schon mehrfach beschriebene Beobachtung, dass mit

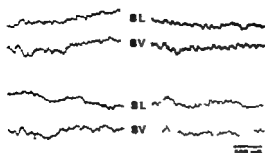


Abb. 3

Abb. 3 Vergleich der gemittelten Potentialkurven des Wortes "Fama" (obere Kurve) und "Mama" (untere Kurve) bei vier verschiedenen Versuchspersonen.

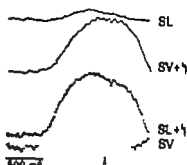


Abb. 4

Abb. 4 Typische Erwartungswellen, ausgelöst durch die Kombination eines sinnvollen und eines sinnlosen Wortes mit einem Lichtblitz (SL, Sinnloser Wortreiz; ST, Sinnvoller Wortreiz)

## ERGEBNISSE

Bei dem ersten Test mit Unterscheidung zwischen nur zwei Worten zeigte sich, dass die evozierten Potentiale nach Anbietung des sinnvollen Wortes grösser und ausgesprägter waren als nach dem sinnlosen phonetischen Äquivalent. Der Grössenunterschied wies zwar eine beträchtliche Streubreite von wenigen bis über 100% auf, konnte jedoch immer erkannt werden (Abb. 3).

Mit einem zusätzlichen Lichtreiz nach jedem sinnvollen Wort konnte eine langsame negative Potentialschwankung im Sinne einer Erwartungswelle ausgelöst werden. Sie trat bei den meisten Fällen deutlich in Erscheinung, bei einigen wenigen Versuchspersonen waren sie nur schwach ausgebildet, aber erkennbar. Diese Erwartungswelle tritt zwischen dem Wort- und Lichtreiz auf. Bietet man den Lichtreiz nach dem sinnlosen phonetischen Äquivalent an, dann kann man gleichfalls eine Erwartungswelle beobachten, wobei in dieser Versuchsanordnung das allein angebotene sinnvolle Wort (untere Kurve) als Vergleichsreiz dient (Abb. 4).

In einigen Versuchen liessen wir nach dem Lichtblitz eine motorische Reaktion ausführen (Handheben). In einer solchen Differenzierungssituation verstärkt diese die Erwartungswelle nicht oder nur ganz unwesentlich (Abb. 5). Unterschiede im Kurvenverlauf betreffen lediglich den Zeitpunkt der motorischen Reaktion selbst, nicht jedoch die Erwartungswelle. In den folgenden Versuchen haben wir daher diese Kombination nicht mehr angewandt.

Bei wiederholter Anbietung von nur einem sinnvollen und einem sinnlosen Wort wäre es möglich, dass die Versuchsperson durch die Wiederholung desselben sinnlosen Wortes dieses als gleichbleibendes Phonem in Erinnerung behält und somit zwischen der phonetischen Charakteristik nicht aber zwischen dem Bedeutungsgehalt beider Worte differenziert. Wir

sehen zwei, acht und dreissig sinnlosen und sinnvollen Worten gezeigt werden konnte. Zur Ausbildung einer Erwartungswelle vor einem Lichtreiz ist es notwendig, dass die Versuchsperson den Warncharakter des dem Lichtsignal vorausgehenden Wortreizes erkennt. Nachdem der Lichtreiz immer nur nach einem bestimmten Reiz, nämlich einem sinnlosen oder sinnvollen Wort angeboten wird, besteht der Warncharakter des Wortreizes in seinem Bedeutungsgehalt. Eine diebezügliche Unterscheidung setzt eine gnostische Verarbeitung und Diskriminierung beider Wortreize voraus. Bei zweikanaliger Registrierung der Potentiale von sinnlosen und sinnvollen Worten mit nachfolgendem Lichtreiz bzw. umgekehrt, stellt daher die Erwartungswelle einen Beweis für die Diskriminationsleistung der Versuchsperson dar. Dabei ist es irrelevant, ob der Lichtreiz nach dem sinnlosen oder sinnvollen Wort angeboten wird: die selektive Auslösung der Erwartungswelle vor dem Lichtreiz beweist die Differenzierung der beiden Wortgruppen mit Zuordnung eines Warncharakters für den dem Lichtreiz vorausgehenden Wortreiz.

Bei ca. 20% der insgesamt 60 Versuchspersonen war trotz normalem Hörvermögen und Sprachverständnisses die Erwartungswelle nur sehr schwach ausgebildet und diagnostisch kaum zu verwerten. In diesem Zusammenhang wäre die Frage zu prüfen, ob in solchen zweifelhaften Fällen durch eine Steigerung der Zahl der angebotenen Testworte bessere Ergebnisse zu erzielen sind. Diebezüglich Erfahrungen an einigen wenigen Fällen sprechen für diese Annahme.

Die Größe der langsamen Hirnpotentialänderungen wird durch die Aufmerksamkeit oder Abwendung der Versuchsperson während des Versuches beeinflusst. Rebert *et al.* (1967) haben darauf hingewiesen und auch wir konnten dieses Phänomen bei schwellennaher Stimulierung beobachten. Dabei muss die Versuchsperson eine wesentlich größere Konzentration aufbringen, um die einzelnen Worte zu diskriminieren. Es ist daher verständlich, dass die Erwartungswelle größer wird als bei überschwelliger Prüfung. Bei einigen schwellennahen Untersuchungen konnten wir allerdings sowohl nach sinnvollen wie auch sinnlosen Worten Erwartungswellen nachweisen, selbst wenn nur einem der beiden Wortreize ein Lichtreiz nachfolgte. Wir fassen dies als Beweis dafür auf, dass die Versuchsperson die beiden Wortgruppen nicht mehr mit Sicherheit unterscheiden konnte und daher auch

einigen Worten jener Gruppe die keinen Warncharakter hatte einen solchen zu ordnen. Damit kam es auch beim Kontrollversuch zur Ausbildung einer Erwartungswelle.

## SUMMARY

In an attempt to develop a method for objective determination of acoustic discrimination, number of meaningful words and nonsensical acoustical equivalents were offered to 70 persons with normal audition. When the meaningful words only were followed by a light flash, a slow negative potential shift (ex-



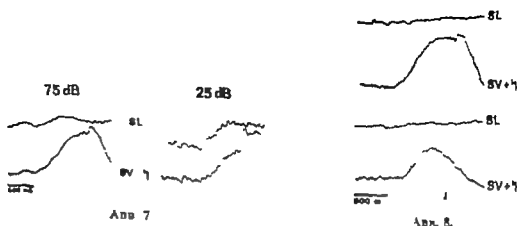


Abb. 7

Abb. 8.

Abb. 7 Vergleich der Erwartungswelle bei 75 dB (Linke Kurvenpaar) und 25 dB (Rechte Kurvenpaar). Während bei überschwelliger Stimulierung nur nach sinnlichem Wort und Lichtblitz eine Erwartungswelle auftritt, findet man eine solche bei schwelennaher Stimulierung auch schon nach dem sinnlosen Wort allein.

Abb. 8 Erwartungswelle nach sinnvollem Wort und Lichtblitz unter Konzentration auf die Reizsituation (oben) und bei Konzentrationsabwendung (unten).

zunehmender Konzentration die Amplitude der Erwartungswelle zunimmt. Bei einigen Versuchspersonen ergab sich allerdings in Schwellennähe auch nach dem sinnlosen Wort, das als Vergleichsreiz allein angeboten wurde, eine deutliche Erwartungswelle (Abb. 7). Dies erscheint uns ein Hinweis darauf, dass die Versuchsperson infolge der Schwellennähe des Reizes den Bedeutungsgehalt der beiden Worte nicht mehr mit Sicherheit differenzieren konnte.

Bei einer Reihe von Versuchspersonen haben wir die Untersuchungen unter konzentrierter Aufmerksamkeit und während des Lesens eines Textes durchgeführt. In den meisten Fällen wurde durch die Konzentrationsabwendung während des Lesens die Erwartungswelle deutlich verkleinert und mitunter auch völlig ausgelöscht (Abb. 8).

## DISKUSSION

Nach Anbietung sinnloser und sinnvoller Worte ohne nachfolgendem Lichtreiz ergeben sich gewisse Unterschiede in den gemittelten Potentialen, wobei diese nach dem sinnvollen Wort meist ein etwas grössere Amplitude aufweisen. Eine ähnliche Beobachtung haben Oswald *et al.* (1960) an Hand der K-Komplexe im EEG beschrieben. Bei Kontrolle eines grösseren Versuchsmaterials zeigt sich jedoch, dass diese Unterschiede kein verlässliches Kriterium für die gnostische Verarbeitung und Differenzierung zwischen den beiden Wortreizen darstellen.

Verwertbar ist in dieser Hinsicht hingegen der Nachweis einer Erwartungswelle, wie dies in den drei Testprogrammen mit Unterscheidung zwel-

## HISTOPATHOLOGICAL CHANGES IN THE AUDITORY PATHWAY IN CASES OF FATAL HEAD INJURY

I. KIRIKAWA, K. EGUCHI, M. OKAMOTO and K. NAKAMURA

*From the Department of Otolaryngology Faculty of Medicine University of  
Tokyo Tokyo Japan*

In five patients who died 24 hours, 3 days, 8 days, a month, and 4 months, respectively after head trauma, the brain and the brain stem were histopathologically examined with special reference to the auditory pathway and the cortex. In one case who survived for 3 days, hemorrhage in the brain stem affected the inferior colliculus, lateral lemniscus, and superior olivary nucleus. In another case who survived for 4 months, multiple softening foci scattered in the brain stem affected also the inferior colliculus, lateral lemniscus, and superior olivary nucleus. In remaining three cases, hemorrhage in varying degrees was observed in the brain stem and the brain. From these findings it was assumed that in case of head trauma, hearing impairment may possibly be caused by retrolabyrinthine lesion.

Although fractures of the skull do not always result from head injuries, varying kinds and degrees of damage occur in the middle ear sound transmission apparatus as well as in the inner ear. Concomitant damage in the intracranial tissues, particularly of the central nervous system and blood vessels, may often occur and is well documented. However hitherto little attention has been paid to the central auditory pathway and its pathological changes due to head injury.

In five cases of fatal head blows of different survival periods, the auditory pathway was studied histopathologically in order to clarify the etiology of hearing loss as a sequelae of the head injury.

### *Clinical Report with Pathology of the Brain and Brain Stem*

In Table I a summary of five cases is made. Age of the patients ranged from 24 to 56. Four cases among five had epi- or subdural hematoma. They lost consciousness and died 24 hours, 3 days, 8 days, a month, and 4 months, respectively after head trauma. Among these cases, two will be discussed in detail.

### *Staining Method of the Central Nervous System*

After fixation in 10% formalin, sliced tissue of the brain and brain stem was embedded in paraffin.

pectancy wave) was found in the time-linked EEG computer analysis prior to one light flash. This result indicates that the expectancy wave can be linked with the word contents, thus enabling an objective criteria of word discrimination.

## RÉSUMÉ

L'audiométrie par analyse de l'EEG mouvement ordinateur permet d'évaluer objectivement les seuils de stimulus sonores, mais ne permet pas de conclure à la compréhension de l'excitation perçue. En comparant les réaction corticales à des stimulus par des mots chargés de sens ou non on constate des variations du tracé conformes à l'information contenue dans l'excitation. On ne trouve une onde d'attente qu'après la perception des mots chargés de sens.

## LITERATUR

- CASPER, H. 1961: Change of cortical H.C. potentials in the sleep-wakefulness cycle. In *The nature of sleep* Churchill, London.
- GILDER, K., V. COHAN, H. G. and COSTA, L. D. 1966: Summated human EEG-potentials with voluntary movement. *Electroenceph. Clin. Neurophysiol.* 20: 433.
- HAINZ, M., GNOLL, E., and STUBBINS, H. 1968: Orientierung- und Bereitschaftspotential bei unerwarteten Reizen. *Exper. Brain Res.* 5: 45.
- KORHUBER, H. H. and DECKE, L. 1965: Hirnpotentialänderung bei Willkürbewegungen und passiven Bewegungen des Menschen. Bereitschaftspotential und reflektorische Potential. *Pflügers Arch. Ges. Physiol.* 384: 1.
- OSWALD, J., TAYLOR, A. M. and TRENKMAN, M. 1960: Discriminative responses to stimulation during human sleep. *B. in* 83: 440.
- REBERT, C. S., McADAM, D. W., KNOTT, J. H. and IRWIN, D. A. 1967: Slow potential change in human brain related to level of motivation. *J. Comp. Physiol. Psychol.* 63: 20.
- SUTTON, S., TURTINO, P., ZUCKER, J. and JOHN, F. R. 1967: Information theory and the sensory evoked potential. *Science* 153: 1436.
- WALTER, W. G., COOPER, R., ALDRIDGE, V. J., McCALL, M. W. C., and WINTER, A. L. 1964: Contingent negative variation: An electrical sign of sensorimotor association and expectancy in the human brain. *Nature* 203.

*Prof. E. Burian, I. Universitätsklinik H.V.O.  
Alserstrasse 4, 1090 Wien, Österreich*



FIG. 1

FIG. 1 (case II) Left hemisphere. Marked edema of the hemisphere, hippocampal herniation and softening of the inferior part of the temporal lobe.

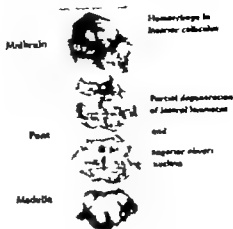


FIG. 2

FIG. 2 (case II) Brain stem. Hemorrhage in the midbrain and the pons.

**Superior olivary nucleus (Fig. 3)** Normally stained ganglion cells and poorly stained ghost-like cells were seen. This partial degeneration was assumed by a pathologist to be a retrograde degeneration secondary to hemorrhage in the inferior colliculus.

**Lateral lemniscus** There was necrotic lesion in the upper part of the pons including medial lemniscus. The nucleus of the lateral lemniscus did not show pathology.

**Inferior colliculus** Hemorrhage in the basal part of the midbrain affected the inferior colliculus.

**Cyrus of Heschl (Fig. 4)** Virchow-Robin's space around the ganglion cell were observed. However it is hard to say whether this finding is pathological.

### Case 1

A 33-year-old man was severely traumatized when he was struck by an automobile. A week later he lost consciousness accompanied by severe vomiting. Contusion of the left leg occurred. A lumbar puncture showed evidence of increased pressure. He was admitted to a hospital with a diagnosis of acute intracranial hemorrhage.

On admission, he was in a state of coma. He responded only to pain stimulation. There were fixed pupils, anisocoria, and choked discs. Examination revealed exaggerated reflexes and Babinski's sign bilaterally. X-ray examination disclosed a fracture line of about 8 cm extending from the parietal region of the base of the skull. Angiography showed upward displacement of the left middle cerebral artery.

A craniotomy resulted in removal of extradural hematoma of the right

TABLE 1 *Pathological changes in the central auditory system*

(—) No pathology — not examined.

No of cases	Age sex	Survival period	Cochl. nucl.	Sup oliv. nucl.	Lat. lem. nucl.	Inf. collic.	Med. gen. body	Heschl. gyrus
I	56, M	24 hrs	(—)	(—)	Hemorrh.	(—)	—	Hemorrh.
II	33 M	3 days	(—)	Degen.	Degen.	Hemorrh.	—	Degen.?
III	24 M	8 days	(—)	Degen.	Degen.	(—)	—	Degen.
IV	39 M	1 mth	(—)	(—)	Hemorrh and degen	—	(—)	Degen.?
V	32, M	4 mths	Softening foci degen	Softening foci, degen.	Softening foci, deg n.	Softening foci, degen	—	Softening foci, degen.

Sections were made in the usual manner. Krüver Barrera's method was employed, which stained the myelin sheath blue, nuclei of the nerve cell and Nissl substance purple. Azan staining was also employed.

### Case II

A 33-year-old man was struck by a falling log which fractured his skull. He was brought to a hospital 10 minutes later. Immediately after admission, he began complaining of parietal headache, vomiting, and losing consciousness. Examination revealed anisocoria and fixed pupils. Craniotomy was performed because extradural hematoma was suspected.

*Operation findings:* There was a marked elevation of intracranial pressure. Extradural hemorrhage covered the frontal, parietal, and temporal regions of the left side. After hematoma was evacuated, a fracture line was observed crossing the anterior branch of the middle meningeal artery near the parietal area, where bleeding still occurred. The temporal lobe and the parietal lobe were markedly compressed by hematoma. Subdural hematoma was noted in the anterior cranial fossa as well as in the middle cranial fossa. Extradural and subdural hematomas weighed 200 g.

The patient remained in coma and died 3 days after operation. The principal findings at autopsy consisted of extradural hematoma, hippocampal herniation of the left side, fracture of the left temporal bone (Fig. 1). There were edema and softening in the brain. Hemorrhagic lesions were observed in the brain stem (Fig. 2).

### *Microscopy of the central auditory pathways*

Ventral cochlear nuclei, dorsal cochlear nuclei, and trapezoid body. No alterations were seen.

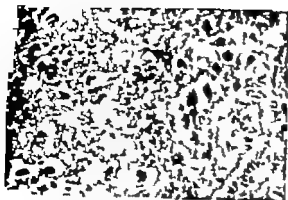


FIG. 3.

FIG. 3 (case 11) Superior olivary nucleus. Small stained ganglion cells and poorly stained, atrophic ganglion cells.

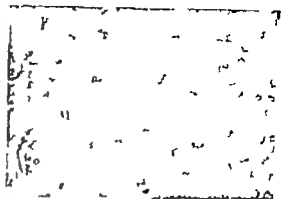


FIG. 4.

FIG. 4 (case 11) Heschl's gyrus. Appearance of Virchow-Robin's space around the ganglion cells.



FIG. 5.

FIG. 5 (case 1) Cross-section of the brain. A softening focus in the inferior surface of the right temporal lobe.

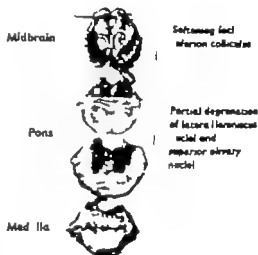


FIG. 6.

FIG. 6 (case 1) Brain stem. Small softening foci in the midbrain and pons.

temporal area (150 g). Following surgery coma persisted. The patient died of anoxia four months after operation.

**Autopsy findings.** Areas of encephalomalacia were seen in the lateral and inferior part of the right temporal lobe and in the inferior part of the left temporal lobe (Fig. 5). Cross section of the brain showed marked subcortical dissemination of lesions of encephalomalacia. The brain stem showed smaller areas of encephalomalacia (Fig. 6).

#### *Microscopy of the central auditory pathways*

**Ventral cochlear nuclei.** No hemorrhage. There was pathology due to softening.



FIG. 7 (case V) Lateral lemniscus nucleus. Some ganglion cells are atrophic and poorly stained. Gliosis and round cell infiltration are visible.

Dorsal cochlear nuclei and trapezoid body: No hemorrhage, no degeneration.

Superior olivary nucleus: No hemorrhage. The cytoplasm of the ganglion cells was poorly stained, suggesting retrograde degeneration.

Lateral lemniscus nucleus (Fig. 7): There was a necrotic lesion involving the nucleus of the lateral lemniscus. Ghost-like and atrophic cells were seen. Demyelinated area was observed. Gliosis and round cell infiltration were also seen.

Inferior colliculus: Small softening area was observed.

Gyrus of Heschl: No alterations were seen.

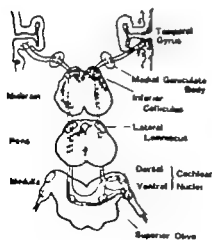


FIG. 8.

FIG. 8. Location of hemorrhage superimposed for cases I-IV.

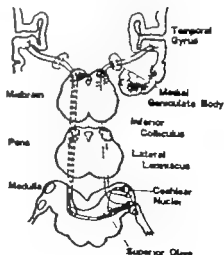


FIG. 9.

FIG. 9. Softening foci (case V).

### Case Analysis

By superimposing maps of hemorrhage of 4 cases, excluding the case which has the softening foci we can see the site of hemorrhage from the brain stem up to the temporal lobe (Fig 8). The map shows that hemorrhage occurs mainly in the midbrain and the pons, particularly in their dorsal part.

Fig 9 is the schematic drawing of site of lesion in the brain and the brain stem in case V who survived 4 months. Small black areas indicate the location of softening foci in the midbrain, pons, and the temporal lobe.

Table 1 illustrates a review of localization of histopathological changes in five cases. It seems that the pathologic change, if present mainly extended from the superior olivary nucleus, through the lateral lemniscus, and up to the inferior colliculus.

### DISCUSSION

Many papers have described histopathology of the brain after injury. Pathology consisted of hemorrhage, edema, degeneration, softening, and necrosis. Pathology may differ in survival periods after injury. Several kinds of changes may coexist in a case.

As to pontine hemorrhage, Altwater (1911) found 12 cases of head injury in 67 cases of pontine hemorrhage. Of these, four had fracture of the base of the skull and hemorrhage in the internal capsule. He stated that pontine hemorrhage was found in cases of severe damage of the parietal area, irrespective of damage of the base of the skull. The pons was not an area of predisposition to hemorrhage. The central area was rather more susceptible to hemorrhage than the periphery in the pons, even secondarily as a result of intracranial pressure elevation.

Wilson & Winkelman (1926) found 13 cases (10%) of pontine hemorrhage in 129 cases of autopsy. Multiple hemorrhagic lesions were present in most of the cases. Extrapontine lesion was present in each case. Wilson found five cases as the result of trauma in 13 cases of pontine hemorrhage although it was not clear when hemorrhage occurred. The pons was indirectly damaged by impact which was transmitted from one hemisphere to the other.

Moore & Stern (1938) postulated that an abrupt pressure change above and down the tentorium caused circulatory disturbance of the basilar artery, then elevated arterial pressure and eventually led to hemorrhage in the pons. Dill & Isenhour (1939) thought that the pontine hemorrhage was due to compression of the pons into the foramen magnum. Extradural hematoma was primarily responsible as it elevated the intracranial pressure.

Beside the direct trauma to the brain stem, the impeding effect of the elevated intracranial pressure to the venous drainage was thought to be the main factor of the brain stem hemorrhage by Poppen *et al* (1952).



Arterial hemorrhage also occurred in the brain stem injury but only occasionally

Areas of predilection to venous hemorrhage in the brain stem was studied by Berner (1940). He investigated anatomy of the vein in the brain stem by intravenous injection of a lead substance, which was photographed by X-ray. There were large veins anterior to the brain stem. The vein in the floor of the fourth ventricle was less vascularized. These findings proposed a problem against the concept that the fourth ventricle was an area of predilection to the hemorrhage. On the other hand, venous hemorrhage was found in the patient who survived more than 48 hours.

Nakamura (1967) found hemorrhagic lesion in the brain stem of a monkey which died instantly by an experimental falling down. The intracranial pressure was measured and proved to be biphasic, negative, and positive.

He stated that the lesions in the brain stem were produced in the following way: an impact distorted the brain within the skull, then the stress caused by distortion concentrated to the brain stem resulting in injury.

The present cases survived and remained unconscious several hours, 3 days, a month, and 4 months, respectively after the injury. This indicated the lesion was extremely severe. Four cases out of five showed extra- and/or subdural hematoma. Besides affection of the cerebrum by pressure of the extradural hematoma, edema, softening (cases II and V), subcortical hemorrhage of the right temporal lobe (case I) and Virchow-Robin's space formation around the ganglion cells in the gyrus of Heschl (cases IV and II) were also observed.

Hemorrhage in the brain stem was considered to be produced by compression of the subdural or extradural hematoma (Dill & Isenhour 1939) or secondarily by herniation of the medial part of the temporal lobe or of the hippocampal gyrus which compressed and distorted the brain stem (Nelson, 1942; Courville, 1945). As a matter of fact, hippocampal herniation was observed in case II. It was hard to decide when hemorrhage occurred in the cases which did not show herniation. In the brain stem, hemorrhage was located in the upper pons (case IV), the inferior colliculus (case II) and the medulla near the outer portion of the posterior area (case I).

The lesion involved the auditory pathway producing degeneration of the lateral lemniscus and its nuclei (cases IV, II, and I) and the inferior colliculus (case II).

Case V is instructive when we consider the pathology of sequelae of head injury. The patient lived 4 months after injury, the longest survival in this series of study. Histology showed small areas of encephalomalacia in the temporal lobes. In the brain stem, cochlear nuclei, the superior olivary nucleus, the lateral lemniscus nucleus, and the inferior colliculus were degenerated by disseminated lesions of softening.

The present cases showed that hemorrhage and degeneration occurred either in central or peripheral area of the brain stem in the gross-sections, although the latter was rather common.

### Case Analysis

By superimposing maps of hemorrhage of 4 cases, excluding the case which has the softening foci we can see the site of hemorrhage from the brain stem up to the temporal lobe (Fig 8). The map shows that hemorrhage occurs mainly in the midbrain and the pons, particularly in their dorsal part.

Fig 9 is the schematic drawing of site of lesion in the brain and the brain stem in case V who survived 4 months. Small black areas indicate the location of softening foci in the midbrain, pons, and the temporal lobe.

Table I illustrates a review of localization of histopathological changes in five cases. It seems that the pathologic change, if present, mainly extended from the superior olivary nucleus, through the lateral lemniscus, and up to the inferior colliculus.

### DISCUSSION

Many papers have described histopathology of the brain after injury. Pathology consisted of hemorrhage, edema, degeneration, softening, and necrosis. Pathology may differ in survival periods after injury. Several kinds of changes may coexist in a case.

As to pontine hemorrhage, Altvater (1911) found 12 cases of head injury in 67 cases of pontine hemorrhage. Of these, four had fracture of the base of the skull and hemorrhage in the internal capsule. He stated that pontine hemorrhage was found in cases of severe damage of the parietal area, irrespective of damage of the base of the skull. The pons was not an area of predisposition to hemorrhage. The central area was rather more susceptible to hemorrhage than the periphery in the pons, even secondarily as a result of intracranial pressure elevation.

Wilson & Winkelmann (1926) found 13 cases (10%) of pontine hemorrhage in 129 cases of autopsy. Multiple hemorrhagic lesions were present in most of the cases. Extrapontine lesion was present in each case. Wilson found five cases as the result of trauma in 13 cases of pontine hemorrhage although it was not clear when hemorrhage occurred. The pons was indirectly damaged by impact which was transmitted from one hemisphere to the other.

Moore & Stern (1938) postulated that an abrupt pressure change above and down the tentorium caused circulatory disturbance of the basilar artery then elevated arterial pressure and eventually led to hemorrhage in the pons. Dill & Isenhour (1939) thought that the pontine hemorrhage was due to compression of the pons into the foramen magnum. Extradural hematoma was primarily responsible as it elevated the intracranial pressure.

Beside the direct trauma to the brain stem the impeding effect of the elevated intracranial pressure to the venous drainage was thought to be the main factor of the brain stem hemorrhage by Poppen *et al* (1952).

Arterial hemorrhage also occurred in the brain stem injury but only occasionally.

Areas of predilection to venous hemorrhage in the brain stem was studied by Berner (1940). He investigated anatomy of the vein in the brain stem by intravenous injection of a lead substance, which was photographed by X-ray. There were large veins anterior to the brain stem. The vein in the floor of the fourth ventricle was less vascularized. These findings proposed a problem against the concept that the fourth ventricle was an area of predilection to the hemorrhage. On the other hand, venous hemorrhage was found in the patient who survived more than 48 hours.

Nakamura (1957) found hemorrhagic lesion in the brain stem of a monkey which died instantly by an experimental falling down. The intracranial pressure was measured and proved to be biphasic, negative, and positive.

He stated that the lesions in the brain stem were produced in the following way: an impact distorted the brain within the skull, then the stress caused by distortion concentrated to the brain stem resulting in injury.

The present cases survived and remained unconscious several hours, 3 days, a month, and 4 months, respectively after the injury. This indicated the lesion was extremely severe. Four cases out of five showed extra- and/or subdural hematoma. Besides affection of the cerebrum by pressure of the extradural hematoma, edema, softening (cases II and V), subcortical hemorrhage of the right temporal lobe (case I) and Virchow Robin's space formation around the ganglion cells in the gyrus of Heschl (cases IV and II) were also observed.

Hemorrhage in the brain stem was considered to be produced by compression of the subdural or extradural hematoma (Dill & Isenhour 1939) or secondarily by herniation of the medial part of the temporal lobe or of the hippocampal gyrus which compressed and distorted the brain stem (Nelson, 1942; Courville, 1945). As a matter of fact, hippocampal herniation was observed in case II. It was hard to decide when hemorrhage occurred in the cases which did not show herniation. In the brain stem, hemorrhage was located in the upper pons (case IV), the inferior colliculus (case II) and the medulla near the outer portion of the posterior area (case I).

The lesion involved the auditory pathway producing degeneration of the lateral lemniscus and its nuclei (cases IV, II, and I) and the inferior colliculus (case II).

Case V is instructive when we consider the pathology of sequelae of head injury. The patient lived 4 months after injury, the longest survival in this series of study. Histology showed small areas of encephalomalacia in the temporal lobes. In the brain stem, cochlear nuclei, the superior olivary nucleus, the lateral lemniscus nucleus, and the inferior colliculus were degenerated by disseminated lesions of softening.

The present cases showed that hemorrhage and degeneration occurred either in central or peripheral area of the brain stem in the cross-sections, although the latter was rather common.

As already described previous papers paid consideration on areas of predilection of brain stem lesions. In our cases, blood vessel distribution in the brain stem was closely related to the location of the lesion. Hemorrhage in the peripheral area accompanied hemorrhagic necrosis in the lateral lemniscus and inferior colliculus.

### ACKNOWLEDGMENTS

The authors wish to acknowledge the contributions of Prof. H. Mannen, Drs. T. Ohno, T. Sato, T. Shitara, Y. Nomura, and members of the research group of otology in our department, all of whom participated in many of these studies.

### RÉSUMÉ

Une étude histopathologique du système nerveux central est faite sur quatre autopsies de blessure fatale de la tête, spécialement à l'égard du cortex et des voies auditives. Il se trouve qu'il y a des régions d'hémorragie et de ramollissement de divers degrés le long des voies auditives, spécialement dans le noyau olivaire supérieur, le colliculus inférieur et le lemniscus latéral. Sur le fondement de l'étude présente une discussion est faite sur la caractéristique de la diminution auditive secondaire à une blessure de la tête.

### ZUSAMMENFASSUNG

Das zentrale Nervensystem wurde in 5 Autopsien von Fällen mit tödlichen Schädelverletzungen histopathologisch untersucht unter besonderer Beachtung der Hörrinde und der akustischen Bahnen. Man fand, dass stellenweise Blutungen und verstreute Erweichungen in unterschiedlichen Stadien entlang der akustischen Bahnen besonders in der oberen Olive, der lateralen Schleife, dem unteren Vierhügel vorhanden waren. Auf Grund der vorliegenden histologischen Untersuchungen hat sich ein Anhalt dafür ergeben, dass wenige Fälle mit dem Kopftrauma die Schädigungen auf die akustischen Bahnen haben und dadurch die zentralen Hörstörung zeigen.

### REFERENCES

- ATTWATER, H. L., 1911 Pontine haemorrhages. *Guy H. p. R. p.* 6: 339.  
 BERNER, O., 1940 A lesion in medulla oblongata and pons. *Verh. Med.* 3: 242.  
 COURVILLE, C. B., 1943 Effect of isolated cranial injury on the midbrain and upper pons. *A. R. S. Soc. Med. Nat. Hist. Proc.* 25: 131.  
 DILL, L. V. and LEXER, C. E., 1939 Etiology of lesions in perimentally produced pontine hemorrhages. *Arch. Neurol. Psychiat.* 41: 1146.  
 KATZ, STEIN, E., 1936 *Das Schädellinnraum*. Schwabe & Co. Basel.  
 MALAMUD, N., 1959 The effect of trauma on the brain stem. *Clin. Neurosurg.* 10: 177.  
 WILKINS, Baltimore.  
 MOORE, M. T. and STERN, R., 1938 Vascular lesion in brain stem and occipital lobe occurring in association with brain tumor. *Brain*, 61: 70.  
 NAKAMURA, N., 1957 Mechanism of head trauma. *Clin. Med.* 9: 1131.  
 NELSON, J., 1942 The mechanism of brain trauma in the presence of subdural hematoma. *J.A.M.A.* 119: 864.

- PORTER, J. L., KRAMER, J. E. JR., and HARRIS, S. P. 1932 Brain stem hemorrhages secondary to supratentorial space-taking lesions. *J. Neuropath., Exp. Neurol.* 11: 287.
- RUBIN, G. 1919: Die Entstehung der pathologisch-anat. mischen Befunde nach Hirnerschütterung in Abhängigkeit vom Gefäßabzweigungssystem des Hirns. *Virchow Arch. Anat.* 226: 134.
- WILSON, G. and WYLLIE, M. W. 1926 Gross pontile bleeding in traumatic and non-traumatic cerebral lesions. *Arch. Neurol. Psychiat.* 15: 485.

I. Kurikae M.D. Dept. of Otolaryngology  
Faculty of Medicine University of  
Tokyo, Bunkyo-ku, Hongo, Tokyo, Japan

## DISCUSSION

J. J. Groen: I have two questions: (1) With what method has he determined the interaural time difference? (2) I have the impression that Mr. Kurikae puts the borderline between normal and abnormal interaural time difference at 60 micro-seconds. We have the experience that none-too-careful, but otherwise normal, listeners need sometimes 90-100 micro-seconds. We would call a pathological case one who needs 200 or more micro-seconds interaural time difference. Would Mr. Kurikae agree upon this with us?

J. McAuliffe, C. M.D.: This very interesting paper deals with a problem which is increasing, namely deafness following head injuries due to automobile accidents. I would like to ask Mr. Kurikae if all the fatal cases had a transverse or longitudinal fracture of the skull? Secondly if any of these patients exhibited a blue tympanum on many occasions. The deafness is often unnoticed by the patient because of the severity of the cerebral injury. Lastly has he observed many cases of spontaneous recovery when the deafness appeared early after accident?

J. Kurikae (Reply): To Mr. Groen: To measure the noticeable time difference we use the earphone method. By changing the distance between two microphones situated in the interaural time difference adjusting apparatus, we can get variable time differences of test tones entering into both ears independently of the intensity difference. Test tone was interrupted band noise (2 times/sec) of 600-800 Hz. We evaluated the value of 0.06 msec as a standard, and when the test result is less than this value, we evaluated it as normal. But, when the test result exceeded this value we suspected the existence of retrocochlear lesion.

To Mr. McAuliffe, C.M.D.: (1) I have several cases of hearing impairment following head trauma who recovered spontaneously. I think it would be possible to recover because in case of unconsciousness it can become conscious if the brain lesion is not organic but rather functional one. When hearing impairment is caused by functional disorders of the auditory organ and the brain itself, it will be possible to recover. However if the lesion in the auditory system is organic, i.e. hemorrhage in the brainstem, even though it is small, hearing impairment will not recover. On the contrary I know a case whose hearing impairment gradually aggravated for 4 months following head trauma. (2) Fractures of the skull were found in three cases out of five autopsied cases. From the observation of survival patients who have a hearing impairment following head trauma, a close relation was found between skull fracture and occurrence of retrocochlear deafness. Fracture of the skull has rather connection with unilateral later onset deafness. (3) Concerning the existence of blue tympanum in our cases, I have no precise data at the present time.

## THE COCHLEA AND THE COCHLEAR NUCLEI IN THE BAT

*Plecotus auritus*

J G HALL,

*From the Anatomical Institution and Rikshospitalet E.N.T. Department  
University of Oslo Oslo Norway*

Twenty specimens of *Plecotus auritus* the most common bat in Norway were collected from an old church tower. Ten animals were used for the preparation of the cochlea the others for the preparation of the brain and the 8th nerve. For the preparation of the cochlea the osmium tetroxide fixation and the surface preparation technique described by H Engström *et al* (1960) was applied.

The brains were fixed in 10% formaline, sectioned in 10 microns and stained with thionine.

The method described by the author (1964) was applied for computing the volume of the cochlear nuclei and for counting their number of nerve cells. Also some data concerning the outer and the middle ear of these animals were collected.

Measurements of the outer ears, the ear-drum, the cochlea, and the basilar membrane were reported. Pictures from these parts of the animals were shown the organ of Corti and the form of the hair cells was discussed. A counting under the microscope showed that 50 of the inner hair cells occupied the same space as 60 in one of the outer rows. It was also shown that the narrow space occupied by the basilar membrane was due to the development of a lamina spiralis ossea secundaria.

The cochlear nuclei were then discussed, their spindle-like form was shown, and their cubic content calculated. Their main sections, the anteroventral, the posteroventral, and the dorsal nucleus were shown in serial square sections. The number of nerve cells of the cochlear nuclei in these bats, 40 000 was compared to the number of cells in the cochlear nuclei of other species employing echo-location especially the whales, and also to the number of nerve cells in the cochlear nuclei of man and cat. The role of the dorsal cochlear nucleus in these species was especially discussed. The distribution of the nerve fibres within the nuclei, shown in a picture prepared by a nerve-fibre staining was compared to the frequency distribution within the nuclei.

### RÉSUMÉ

L'oreille externe et interne chez le chauve-souris est décrite ainsi que les noyaux cochléaires. L'auteur a collectionné 20 exemplaires provenant d'une vieille tour d'église en Norvège. Dix exemplaires ont été utilisés pour préparer la cochlée d'après la méthode de H Engström le reste a été utilisé pour préparer les noyaux cochléaires dans le bulbe, d'après la méthode que l'auteur a décrit en 1964.

## ZUSAMMENFASSUNG

Das äussere und innere Ohr der Fledermäuse, *Plecotus auritus*, wird beschrieben, sowie die Cochlearkerne derselben. Der Verfasser hat 20 Exemplare in einem alten Kirchthurm in Norwegen gesammelt. Von 10 Exemplaren sind die Cochleae nach der Methode von H. Engström präpariert, in den anderen 10 sind die Medullae nach der von dem Verfasser in 1964 beschriebenen Methode präpariert worden.

## REFERENCES

- ENGSTROM, H. 1964. *Acta Anat.* 172 pp. Almqvist & Wiksell, Stockholm.  
 GRAYBI, D. R., 1966. *Living in the dark*, 413 pp. Yale University Press.  
 HALL, J. G., 1964. The cochlea and the cochlear nuclei in neonatal *Myotis*. *Acta Otolaryng.* (Stockh.) 5 ppl. 195.  
 — 1967. The ring and primary auditory centres of the whale. *Acta Otolaryng.* (Stockh.) 5 ppl. 223.  
 OERTL, K., 1966. Personal communication.  
 PIZ, A., 1966. The megachiroptera and lesser chiroptera of the microchiroptera. The structure of the cochlea in chiroptera. *J. Morph.*, 118: 445.  
 PIZ, J. D., 1961. Echolocation by bats. *Endocrinol.* 19, 78: 161.  
 ROSE, J. E., GALT, R., and H. C. J., 1959. Microelectrode studies of the cochlear nuclei in the cat. *Johns Hopkins Bull.* 18: 211.

The Anatomical Institution and Rikshospitalet  
 S-141 Dept University of Gote, Goteborg  
 Norway

## DISCUSSION

G. d. W. In whales there is intense interconnection between the right and the left cochlear nuclei, even so intense that it is already microscopically visible. Is this the same case in bats?

J. G. Hall (Reply) to Mr G. d. W. It is true that the corpus trapezoidum is present also in the flying bats, but the fibres do not cross from the cochlear nuclei on the one side to the other but traverse a distance to the lateral lemniscus on the other side.

Owing to the number of illustrations belonging to this work, the article could not be included in this issue but will be published, in its full version, in number 3 of the journal.

## THE COCHLEA AND THE COCHLEAR NUCLEI IN THE BAT

### *Plecotus auritus*

J G HALI

*From the Anatomical Institution and Rikshospitalet E.A.T. Department  
University of Oslo Oslo Norway*

Twenty specimens of *Plecotus auritus* the most common bat in Norway were collected from an old church tower. Ten animals were used for the preparation of the cochlea, the others for the preparation of the brain and the 8th nerve. For the preparation of the cochlea the osmium tetroxide fixation and the surface preparation technique described by H Engström *et al* (1966) was applied.

The brains were fixed in 10% formaline, sectioned in 10 microns and stained with thionine.

The method described by the author (1964) was applied for computing the volume of the cochlear nuclei and for counting their number of nerve cells. Also some data concerning the outer and the middle ear of these animals were collected.

Measurements of the outer ears, the ear-drum, the cochlea, and the basilar membrane were reported. Pictures from these parts of the animals were shown, the organ of Corti and the form of the hair cells was discussed. A counting under the microscope showed that 50 of the inner hair cells occupied the same space as 60 in one of the outer rows. It was also shown that the narrow space occupied by the basilar membrane was due to the development of a lamina spiralis ossea secundaria.

The cochlear nuclei were then discussed, their spindle-like form was shown and their cubic content calculated. Their main sections, the anteroventral, the posteroventral, and the dorsal nucleus were shown in serial square sections. The number of nerve cells of the cochlear nuclei in these bats, 40 000, was compared to the number of cells in the cochlear nuclei of other species employing echo-location, especially the whales, and also to the number of nerve cells in the cochlear nuclei of man and cat. The role of the dorsal cochlear nucleus in these species was especially discussed. The distribution of the nerve fibres within the nuclei, shown in a picture prepared by a nerve fibre-staining, was compared to the frequency distribution within the nuclei.

### RÉSUMÉ

L'oreille externe et interne chez la chauve-souris est décrite ainsi que les noyaux cochléaires. L'auteur a collectionné 20 exemplaires provenant d'une vieille tour d'église en Norvège. Dix exemplaires ont été utilisés pour préparer la cochlée, d'après la méthode de H Engström, le reste a été utilisé pour préparer les noyaux cochléaires dans le bulbe, d'après la méthode que l'auteur a décrite en 1964.



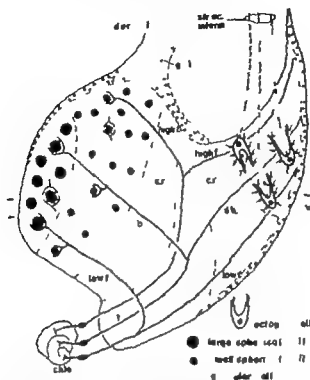


FIG. 1. Diagram of the cat ventral cochlear nucleus in the sagittal plane. The bifurcated and tonotopic organization of the cochlear nuclei fibers (*f*) are shown. Except for the superficial layer of granular cell fibers (*g.c.f.*) only the distribution and type of fiber of the fiber cell types under consideration are indicated. For further explanation, see the text. *a.b.* ascending cochlear branches; *c.n.* central region; *d.b.* descending cochlear branches; *high f* high tonal frequencies; *low f* low tonal frequencies; *str. ac. intern.*, intermediate acoustic stria.

cochlea projects ventrally and the basal part dorsally (Fig. 1). Accordingly in the cochlear nuclei high tonal frequencies are represented dorsally or above and low frequencies ventrally or below. The course of the ascending and descending cochlear branches differs fundamentally: the ascending branches tend to diverge while the descending ones are distinctly convergent.

The cells in the anterior part of the ventral cochlear nucleus appear round without visible dendrites in Nissl sections, and I have therefore called them pherical cells. According to their size I have classified them as two types, viz., large and small pherical cells. In Nissl preparations these two cell types appear much alike: they are both characterized by a peculiar arrangement of the Nissl substance which forms a distinct nuclear cap and a concentric ring of coarse granules (Fig. 2*A* & *B*). Both types are supplied from the descending cochlear branches by means of typical bulbs of Held (Fig. 3*A* & *B*) which represent a special type of synaptic endings (Held,

## THE INTRINSIC ORGANIZATION OF THE COCHLEAR NUCLEI IN THE CAT

KIRSTEN KJLLSBERG ØSEN

*From the Anatomical Institute University of Oslo Oslo Norway*

In the cochlear nuclei nine different cell types are distinguished on the basis of Nissl preparations. Only three of these cell types are presented here. *The large and small spherical cells* are innervated from the ascending cochlear branches by means of typical bulbs of Held. The former cell group is apparently supplied only from the apical and middle part of the cochlea and projects bilaterally on the medial superior olive, while the latter group seems to be supplied from the entire cochlea and projects on the lateral superior olive on the same side. *The octopus cells* are innervated from the descending cochlear branches by means of small ring shaped boutons. The axons of these cells run in the intermediate acoustic stria and terminate probably in the retroolivary and medial preolivary nuclei on both sides. The function of the three types of cells is discussed.

The cochlear nuclei form the first relay center in the ascending auditory pathway. In these nuclei each cochlear nerve fiber establishes contact with several different types of cells, most of which project further centrally. Thus the impulses which are set up in the cochlea become transferred into different paths leading up to higher auditory centers. Although these paths may be integrated at all levels, the organization of the cochlear nuclei presumably provides a key to a better understanding of the entire auditory system.

On the basis of Nissl preparations nine different cell types may be distinguished in the cochlear nuclei of the cat and I have proposed a parcellation of the entire complex based exclusively upon the distribution of these cells. A satisfactory description of all cell types would be impossible in a quarter of an hour and therefore only three of them will be presented here.

In mammals the cochlear nuclei consist of a ventral and dorsal nucleus. The cells in question viz., the large and small spherical cells and the octopus cells, occupy the most anterior and posterior parts of the ventral nucleus as shown in Fig. 1. The central region of this nucleus contains a mixture of cell types which are not being commented upon here.

According to the Golgi studies of Cajal (1909) and Lorente de Nó (1933) all cochlear nerve fibers divide into ascending and descending branches. As shown anatomically by Sando (1965) and physiologically by Rose *et al* (1969) the branches are arranged tonotopically so that the apical part of the

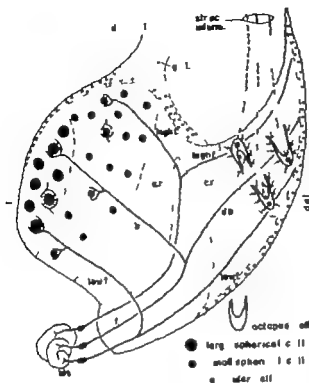


FIG. 1. Diagram of the cat ventral cochlear nucleus in the sagittal plane. The bifurcation and tonotopical organization of the cochlear nerve fibers (*f*) are shown. Except for the superficial layer of granular cells (*g.c.*) only the distribution and type of innervation of the three cell types under consideration are indicated. For further explanation, see the text. *db*: descending cochlear branches; *cr*: central region; *high f*: high tonal frequencies; *low f*: low tonal frequencies; *del*: dendrites; *straculum*: straculum.

cochlea projects ventrally and the basal part dorsally (Fig. 1). Accordingly in the cochlear nuclei high tonal frequencies are represented dorsally or above and low frequencies ventrally or below. The course of the ascending and descending cochlear branches differs fundamentally: the ascending branches tend to diverge while the descending ones are distinctly convergent.

The cells in the anterior part of the ventral cochlear nucleus appear round without visible dendrites in Nissl sections, and I have therefore called them spherical cells. According to their size I have classified them as two types, i.e., large and small spherical cells. In Nissl preparations these two cell types appear much alike: they are both characterized by a peculiar arrangement of the Nissl substance which forms a distinct nuclear cap and a concentric ring of coarse granules (Fig. 2A, B). Both types are supplied from the ascending cochlear branches by means of typical bulbs of Held (Fig. 3A, B) which represent a special type of synaptic endings (Held,

## THE INTRINSIC ORGANIZATION OF THE COCHLEAR NUCLEI IN THE CAT

KIRSTEN KJELSDERG OSEN

*From the Anatomical Institute University of Oslo Oslo Norway*

In the cochlear nuclei nine different cell types are distinguished on the basis of Nissl preparations. Only three of these cell types are presented here. *The large and small spherical cells* are innervated from the ascending cochlear branches by means of typical bulbs of Held. The former cell group is apparently supplied only from the apical and middle part of the cochlea and projects bilaterally on the medial superior olive, while the latter group seems to be supplied from the entire cochlea and projects on the lateral superior olive on the same side. *The octopus cells* are innervated from the descending cochlear branches by means of small ring shaped boutons. The axons of these cells run in the inter-mediate acoustic stria and terminate probably in the retroolivary and medial preolivary nuclei on both sides. The function of the three types of cells is discussed.

The cochlear nuclei form the first relay center in the ascending auditory pathway. In these nuclei each cochlear nerve fiber establishes contact with several different types of cells, most of which project further centrally. Thus the impulses which are set up in the cochlea become transferred into different paths leading up to higher auditory centers. Although these paths may be integrated at all levels, the organization of the cochlear nuclei presumably provides a key to a better understanding of the entire auditory system.

On the basis of Nissl preparations nine different cell types may be distinguished in the cochlear nuclei of the cat, and I have proposed a parcellation of the entire complex based exclusively upon the distribution of these cells. A satisfactory description of all cell types would be impossible in a quarter of an hour and therefore only three of them will be presented here.

In mammals the cochlear nuclei consist of a ventral and dorsal nucleus. The cells in question viz. the large and small spherical cells and the octopus cells, occupy the most anterior and posterior parts of the ventral nucleus as shown in Fig. 1. The central region of this nucleus contains a mixture of cell types which are not being commented upon here.

According to the Golgi studies of Cajal (1909) and Lorente de Nó (1933) all cochlear nerve fibers divide into ascending and descending branches. As shown anatomically by Sando (1965) and physiologically by Rose *et al* (1959) the branches are arranged tonotopically so that the apical part of the

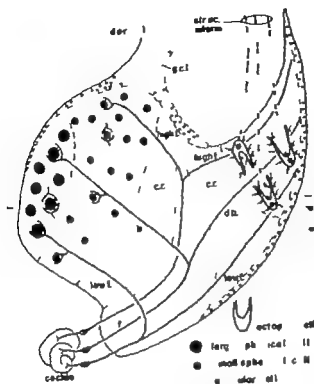


FIG. 1 Diagram of the cat central cochlear nucleus in the sagittal plane. The bifurcation and tonotopical organization of the cochlear nerve fibers (cf) are shown. Except for the superficial layer of granular cells (g.c.l.) only the distribution and type of innervation of the three cell types under consideration are indicated. For further explanation, see the text. *h* ascending cochlear branches *ec* central region *db* descending cochlear branches; *high f* high tonal frequency nuclei *low f* low tonal frequencies (*ec. int. r.* intermediate acoustic stria).

cochlea projects ventrally and the basal part dorsally (Fig. 1). Accordingly in the cochlear nuclei high tonal frequencies are represented dorsally or above and low frequencies ventrally or below. The course of the ascending and descending cochlear branches differs fundamentally: the ascending branches tend to diverge while the descending ones are distinctly convergent.

The cells in the anterior part of the central cochlear nucleus appear round without visible dendrites in Nissl sections, and I have therefore called them ph. cells. According to their size I have classified them as two types, viz., large and small ph. cells. In Nissl preparations these two cell types appear much like they are both characterized by a peculiar arrangement of the Nissl substance which forms a distinct nuclear cap and a concentric ring of coarse granules (Fig. 2 & B). Both types are supplied from the ascending cochlear branches by means of typical bulbs of Held (Fig. 3 & B) which represent a special type of synaptic endings (Held,



FIG. 2. Photomicrographs of large spherical cells (A) small spherical cell (B) and an octopus cell (C) 15  $\mu$  section Nissl stain 480

1893 Cajal 1900) The cell bodies are usually embraced by two or three bulbs which probably means that only a few cochlear nerve fibers converge upon each cell. Also the large number of these cells, as compared with the other cell types of the cochlear nuclei, is compatible with a slight degree of convergence of primary afferents in this region.

As shown in Fig. 1 the large spherical cells are densely packed in a cap-shaped area which does not reach the dorsalmost part of the nucleus. These cells, therefore, are probably concerned merely with the conduction of low and middle tonal frequencies. The small spherical cells, on the other hand, form a broad band of cells which crosses all the ascending branches from high to low and thus may be engaged in the conduction of all frequencies.

Despite their similar appearance the large and small spherical cells definitely have different central connections. I have studied the course of their axons by means of anterograde degeneration following restricted lesions of the cochlear nuclei. The lesions were made stereotactically by electrocoagulation, and the sections were stained by the silver methods of Gies

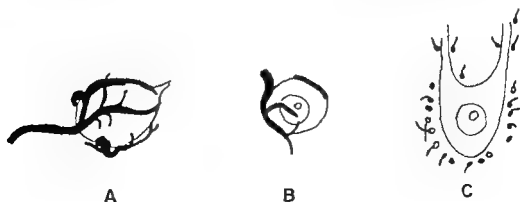


FIG. 3. Drawing of bulb of Nissl applying large spherical cell (A) and small spherical cell (B) and ring shaped boutons applying an octopus cell (C) 15  $\mu$  section stained by the method of Gies

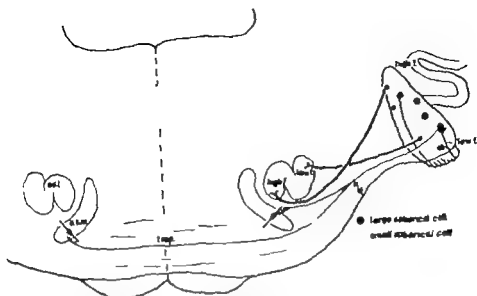


FIG. 4. Diagram of the medulla in the transverse plane showing the location and projection of the large and small spherical cells. The direction of the large cell axis, indicated here, has not been proved. For further explanation, see the text. *co. trap.*, Trapezoid body; *high f*, high tonal frequencies; *low f*, low tonal frequencies; *l.s.o.*, lateral superior olive; *m.s.o.*, medial superior olive.

and Nauta. This technique makes it possible to follow the degenerated nerve fibers from the place of lesion to their site of termination. In all 30 cats were used in these experiments and from a comparison of a great number of cases it appeared that the large spherical cells project bilaterally upon the medial superior olive while the small ones project on the lateral superior olive on the same side (Fig. 4). Only the main projection of the cells is considered here while possible additional projections to other nuclei are left out of account.

The cells of the medial superior olive are bipolar and arranged in the transverse plane. Each cell is innervated bilaterally from the cochlear nuclei: the right dendrites from the right side and the left dendrites from the left side. Slotter showed this in 1953 but he did not recognize from which part of the cochlear nuclei these fibers originated. As each cell of the medial superior olive receives impulses from both ears, physiologists have suggested that this nucleus is concerned in the localization of sound. More particularly the medial superior olive may be involved in the sound-eye reflexes as it, according to the comparative anatomical study of Irving & Harrison (1967) is especially well developed in animals with large eyes. On the other hand, it is definitely not involved in the echolocation mechanism of the porpoise and bat as in these animals both the medial superior olive and the large spherical cells are rudimentary. The fact that these animals are mainly adapted for ultrasonics supports my suggestion that



Fig. 3 Photomicrograph of the octopus cell area (oca) with the adjacent parts of the ventral portion of the ventral cochlear nucleus (c) and the granular cell layer (gcl). The critical line indicates the border between the oca and the c. The dendrites of the octopus cells are oriented vertically across the direction of the unstained descending cochlear branches, the direction of which is indicated by arrow 50. The section is stained for acetylcholinesterase according to a modification of the thiocholin method of Koelle & Friedenwald. Incubation with acetylthiocholine for 2 hours. Inhibitor Mipa.  $\times 110$ .

this portion of the auditory system is operated mainly by low tonal frequencies.

The small spherical cells project on the lateral superior olive in a strict tonotopical manner: the dorsal part of the area projects medially and the ventral part laterally as shown in Fig. 4. Accordingly the lateral superior olive should be tonotopically organized so that low tonal frequencies are represented laterally and high frequencies medially and that is exactly what was found physiologically by Tsuchitani & Boudreau (1960) in the cat. According to their findings, moreover all tonal frequencies audible to a cat are represented in the lateral superior olive, an observation which is highly compatible with my suggestion that the small spherical cells are supplied from the entire cochlea. The strict tonotopical organization, the low degree of convergence, and the relation to all frequencies apparently make this portion of the auditory system especially well suited for frequency analysis. Interestingly both the area of small spherical cells and the lateral superior olive are remarkably large in the porpoise which is capable of hearing tones as high as 150 kc/s or more.



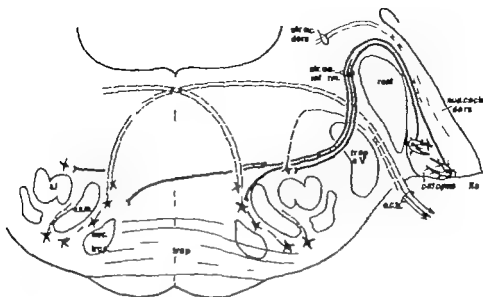


FIG. 6. Diagram of the medulla in the transverse plane showing the location and lateral projection of the octopus cells. The retrochiasmatic and medial prechiasmatic nuclei are indicated as black cells. For further explanation, see the text. *cc* = cerebellar body; *tr. p.* = trapezoid body; *tr. p.* = trapezoid nucleus; *ac. coch. dors.* = dorsal cochlear nucleus; *a.c.b.* = olivocochlear bundle; *a.s.l.* = lateral superior olive; *a.s.m.* = medial superior olive; *ac. dors.* = dorsal acoustic stria; *ac. vent. med.* = medial acoustic stria; *trap. 5* = spinal fifth tract.

The octopus cells are situated in the caudalmost part of the ventral cochlear nucleus (Fig. 1). In contrast to the spherical cells they are characterized by their thick, straight dendrites which course vertically across the direction of the descending cochlear branches. Due to the arrangement of their dendrites these cells often look like octopuses and therefore I proposed that name for them. In *Wassal* sections their perikarya show very fine *Wassal* granules and only the roots of the dendrites are visible (Fig. 2C). The dendritic pattern becomes more conspicuous in sections stained for acetylcholinesterase according to the *Hoelle* method, as seen in Fig. 5. The same cells have also been described by *Harrison & Irving* (1966) who classified them as *k*-cells. As the octopus cells are situated at the site where the descending cochlear branches are most closely packed the dendrites of each cell evidently cross a great number of branches. Both the perikarya and the dendrites are innervated from these branches by means of small ring-shaped boutons (Fig. 3C). Though it remains to be definitely proved, the anatomical features suggest that each octopus cell, in sharp contrast to the spherical cells, is innervated from a great number of cochlear nerve fibers. The small number of octopus cells also favours the assumption that a considerable convergence of primary afferents takes place upon them.

Although the central projection of the octopus cells is not defined in all details, there exists some evidence for the main course of their axons. From



FIG. 5. Photomicrograph of the octopus cell area (oca) with the adjacent parts of the central region of the ventral cochlear nucleus (cr) and the granular cell layer (gcl). The critical line indicates the border between the oca and the cr. The dendrites of the octopus cells are oriented critically across the direction of the unstained descending cochlear branches, the direction of which is indicated by arrow. 50  $\mu$  sagittal section stained for a cholinergic trace according to a modification of the thiocholine method of Koell & Fledermann. Incubation with acetylthiocholine for 2 hours. Inhibitor Mipa.  $\times 110$ .

this portion of the auditory system is operated mainly by low tonal frequencies.

The small spherical cells project on the lateral superior olive in a strict tonotopic manner: the dorsal part of the area projects medially and the ventral part laterally as shown in Fig. 4. Accordingly the lateral superior olive should be tonotopically organized so that low tonal frequencies are represented laterally and high frequencies medially, and that is exactly what was found physiologically by Tauchnitz & Boudreau (1966) in the cat. According to their findings, moreover, all tonal frequencies audible to a cat are represented in the lateral superior olive, an observation which is highly compatible with my suggestion that the small spherical cells are supplied from the entire cochlea. The strict tonotopic organization, the low degree of convergence, and the relation to all frequencies apparently make this portion of the auditory system especially well suited for frequency analysis. Interestingly both the area of small spherical cells and the lateral superior olive are remarkably large in the porpoise which is capable of hearing tones as high as 150 kc/s or more.

- HESS, H., 1923 Die centrale Gehörleitung. *Arch Anat Physiol* 201
- LEVINE, R., and HARRISON, J. M., 1967 The superior olivary complex and its relation to the auditory system. A comparative study. *J Comp. Neurol* 130 77
- LOEFGREN, R., 1933 Anatomy of the eighth nerve. *Laryngoscope* 43 1
- MACLEOD, G. L., 1946 The olivary peduncle and other fiber projections of the superior olivary complex. *J Comp. Neurol.* 88 141
- ROSE, J. E., GALAMBOS, R. and HENNING, J. H. 1959 Microelectrode studies of the cochlear nuclei of the cat. *Bull J Am Hlth Hosp.* 106 211
- SANO, L., 1965 The anatomical relationships of the cochlear nerve fibers. *Acta Otolaryng* (Stockh.) 68 417
- STOTLER, W. A., 1953 A experimental study of the cells and connections of the superior olivary complex of the cat. *J Comp Neurol* 98, 401
- TRICHTER, C., and BOTDANOV, J. C., 1966 Single unit analysis of cat superior olive S segment with tonal stimuli. *J Acoust Physiol* 29 684

Anatomical Institute  
University of Oslo  
Oslo, Norway

my own experimental studies in kittens it appeared that the octopus cells project centrally via the intermediate acoustic stria (Fig 6) a few days after the stria was cut the octopus cells showed definite signs of acute retrograde degeneration. In these experiments the stria was cut just medial to the restiform body and the course of the axons could not be determined beyond this point. However the central course of the intermediate acoustic stria was recently shown by Fernández & Karapas (1967) in experimental anatomical studies on adult cats. They found that the fibers terminate bilaterally in the retroolivary and medial preolivary nuclei (Fig 6). As these nuclei give origin to the olivocochlear bundle of Rasmussen (1946) one may suggest a relation between the octopus cells and the bundle. Though it is not definitely proved, it seems reasonable that there exists here a short reflex arch composed of three links, namely the spiral ganglion cells, the octopus cells, and the cells of the retroolivary and medial preolivary nuclei. The presumed convergence of auditory impulses upon the octopus cells apparently makes them particularly well suited to form a link in this inhibitory feed back mechanism.

#### RÉSUMÉ

Une division des noyaux cochléaires est proposée. Elle est fondée sur la séparation microscopique de neuf groupes cellulaires différents. Parmi ceux-ci, trois groupes sont décrits au point de vue de leur rôle possible dans l'audition.

#### ZUSAMMENFASSUNG

In den Cochlearis-kernen der Katze lassen sich in Nissl-Präparaten neun verschiedene Zelltypen unterscheiden von denen drei hier besprochen werden. Aufsteigende Cochleariskerne innervieren die grossen und kleinen sphärischen Zellen unter Bildung der typischen Heldschen kolbenartigen Anschwellungen. Die grossen Zellen werden offenbar nur vom apikalen und mittleren Teil der Cochlea versorgt und sie projizieren bilateral auf die mediale obere Olive. Die kleinen Zellen scheinen von der ganzen Cochlea versorgt zu werden und projizieren auf die laterale obere Olive der gleichen Seite. Die Octopusellen werden von absteigenden Cochleariskernen unter Bildung von kleinen, ringförmigen Boutons innerviert. Die Axone der Octopuszellen verlaufen in der Stria acustica intermedia und enden wahrscheinlich bilateral in retroolivaren und medialen präolivaren Kernen. Die Funktion der drei besprochenen Zelltypen wird diskutiert.

#### REFERENCES

- CARRI, S. R. 1909: *Histologie du système nerveux de l'homme et des mammifères*, chap. 23, p. 774. A. Maloine Paris.
- FERNÁNDEZ, C., and KARAPAS, A. F. 1967: The course and termination of the striae of M. low and Held in the cat. *J. Comp. Neurol.* 131: 371.
- HARRISON, J. M., and LARSEN, R. 1966: The organization of the posterior central cochlear nucleus in the rat. *J. Comp. Neurol.* 126: 391.



Fig. 1. 1st phase vestibular imbalance after the introduction of NaCl-saturated solution into the middle ear on the left side 1st phase "irritative" type.

Fig. 2. Biphasic vestibular imbalance after the introduction of NaCl-saturated solution into the middle ear on the left side 2nd phase "pathologic" type.

ion and molecules. This exchange ought to be regulated also by the osmotic or hydrodynamic pressure.

The introduction of a saturated solution of NaCl (0.2 ml) into the middle ear of a guinea pig caused an acute biphasic vestibular imbalance. The experimental animals showed, a few minutes after the injection, a vestibular picture of the "irritative" type of nystagmus in relation to the injected side, and tonic deviation of the head, trunk, and limbs towards the other side.

## MODIFICATIONS OF THE OSMOTIC PRESSURE OF PERILYMPH AND ENDOLYMPH

*An Hypothesis on the Pathogenesis of Menière's disease*<sup>1</sup>

M. ARSLAN

*From the E.N.T. Department University of Padua, Padua, Italy*

The introduction of a saturated solution of NaCl into the middle ear of a cat or guinea pig provoked a biphasic vestibular spontaneous picture which is composed of a 1st phase of "irritative" signs (nystagmus beating towards the injected side etc.) and of a 2nd phase of "paralytic" signs (nystagmus beating towards the non injected side, etc). Histological, electrophysiological, biochemical, and biophysical (osmotic pressure measurements) investigations have been carried out in many series of experiments performed by the author and his collaborators. It has been demonstrated that the changes of osmotic pressure between the perilymph and the endolymph are the essential pathogenetic factors of all clinical histological, and electrophysiological manifestations occurring in the experiment. Close analogies are indicated between the effects of the experiment and the pathogenetic factors and evolution of Menière's acute attacks: in fact a Menière's attack shows "irritative" signs followed by "paralytic" signs. Furthermore, in these experiments and in a Menière's attack there are identical histological pictures of a "collapse" of the endolymphatic duct.

It is generally admitted that pathologic modifications of secretory and biochemical mechanisms regulating the perilymphatic and endolymphatic circulation are the basic pathogenetic factors of labyrinth hydrops (Menière's disease). The continuous and precise processes of the reciprocal regulation of the perilymphatic and endolymphatic circulation depend (1) on the secretion and absorption mechanisms of the two liquids, and (2) on the permeability of the labyrinth membranes and structures. As many authors have demonstrated (Altmann & Waltner 1950; Rauch, 1966; Tonndorf 1957 etc.) exchange of water ions, and other substances between perilymph and endolymph appears highly possible, as their chemical characteristics are completely different. In fact the separating membranes, such as Reissner's membrane and other membranes, may be passed through by water.

This paper is a synthesis of a large number of experimental studies of different branches (histologic, biochemical, biophysical, etc.). The investigations were carried out mainly by Dr G. Minari, M.D. with the collaboration of Dr G. Sala, M.D., Dr F. Giacomini, M.D., G. Perin, Ph.D. and Dr W. Morso, M.D. all of whom are members of the E.N.T. Dept. of the University of Padua.

phalic duct and modifies the functional conditions of the receptors (vestibular "irritative signs")

5 Compensation mechanisms in the secretory structures of the stria vascularis and perilymphatic walls (such as a rapid increase of the ultrafiltration of water) overturn the vestibular imbalance (vestibular "paralytic" signs) and, finally reestablish in about 1-2 hours the osmotic balance and, consequently the functional state of the receptors, which return to normality. Fig. 3 is a schematic drawing of this hypothesis.

In order to prove in an objective way the validity of these hypothesis, we first had to demonstrate the semipermeability of the round window membrane that is, the passage through it of the liquid (perilymph) having lesser osmotic pressure to the liquid of the middle ear having greater osmotic pressure (hypertonic solution).

Various-sized NaCl crystals (mol. weight 58.46, high constancy of electrolytical dissociation, stable neutral pH) were applied on the round window of anesthetized cats (sodium pentobarbital, 50 mg/kg intraperitoneally) while in series of control animals crystals of methylene blue were employed.

*After the Deposition of an NaCl Crystal on the Round Window Membrane (Fig. 4) we could observe*

1. Appearance of perilymph on the membrane in 15 to 18 seconds.
2. Dissolution of crystal and progressive reduction in size.
3. Complete filling of the fossa of the round window with perilymph in nearly 3 minutes, so that the crystal was no longer visible.

As an added proof of the suggestion of H. Sponddin, the histological effects of the deposition of NaCl crystals on the round window was studied by G. Babighian of our department. The round window bilateral membranes of cats were exposed in the retroauricular approach and the opening of the bullae. On the membrane of one side sodium chloride crystal was deposited (the contralateral membrane served as control). Crystal dissolution caused water to leave the perilymph; water was removed after complete dissolution of the NaCl crystal and the round window membrane was imbedded in paraffin (stained with haematoxylin-eosin). We observed the following histologic modifications (Fig. 5).

The low cubic epithelium lining the round window tympanic wall appeared reactivated and disarranged, so that its morphologic detail were hardly identifiable. By some preparations, cells appeared detached from the basement membrane.

The fibrous connective layer which supports the round window membrane appeared markedly edematous, the interstitial fluid was collected in small spaces and bits of the tissue enlarging it. Moreover some slit were partly filled with homogeneous, lightly acidophilic material. We never observed phagocytic cells nor nuclear damage of the fibroblasts. The mesothelial cells were washed out, but there was no other pathological aspect (Fig. 6 shows the contralateral control round window membrane (normal)).

We state that the passage of perilymph to the round window and perhaps of tears, too, from the scala tympani to the middle ear, like the semipermeable mechanism, occurring in the round window membrane is made possible by structural lesions induced from high hypertonic solution formed after NaCl crystal deposition.

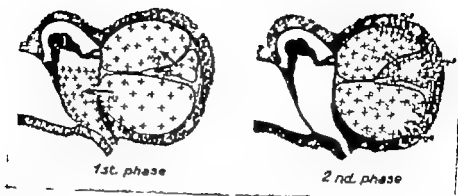


FIG. 3. Schematic representation of the experiment 1st phase Water passes from the perilymphatic space to the middle ear owing to the different osmotic pressures. From the endolymph, too, there is probably a passage of water to the perilymph and consequently the endolymphatic canal is compressed and collapsed. 2nd phase After osmotic equilibration between the middle ear solution and the perilymph, water loss of the perilymph is quickly compensated by the ultrafiltration mechanism, and so osmotic difference arises between perilymph and endolymph, which is now hypertonic. Consequently water passes from the perilymph to the endolymph, so that the endolymphatic canal bulges.

These phenomena lasted from 15 to 30 min. In the second phase of the experiments, beginning 40-50 min after the injection, the same vestibular signs reappeared but in the opposite direction ("paralytic" type) lasting from 60 to 90 min (Figs. 1 and 2).

Nearly 2 hours after the injection, animals no longer presented signs of labyrinthine disfunction and they returned to a perfect normality as a matter of fact thermic stimulation of the labyrinth of the injected ear at this moment gave values of provoked nystagmus equal to those obtained before the experiment (normal reflectivity) (Arslan, 1934).

The hypothesis formulated by us for the interpretation of the mechanisms of this experimental finding was as follows:

1 The introduction of hypertonic saline solution into the middle ear produces in this cavity an high osmotic pressure. As is known, hypertonic or saturated solutions injected into mucous-lined cavities are slowly diluted by water drawn from the interstices of the mucous membrane and its cells and vessels.

2 High osmotic pressure in the middle ear could provoke a passage of perilymph through the round window from the perilymphatic spaces to the middle ear.

3 This passage of water is made possible because the round window membrane became a semipermeable membrane.

4 The loss of water inside the perilymph provokes an acute crisis in the osmotic balance between the perilymph and the endolymph which probably causes a passage of water from the endolymph to perilymph through Reissner's membrane. This fact is responsible for the collapse of the endolymph.



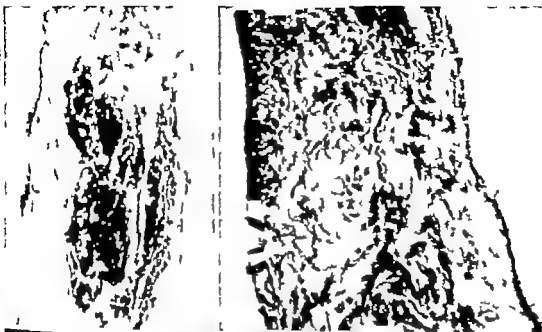


FIG. 5 Round window membrane (cat) after position of crystal for 5 minutes.

FIG. 6 Normal round window membrane (cat) (control)

FIG. 9 Collapsed endolymphatic duct (1st phase of the experiment). Irritable vestibular signs. The cupula appears compressed on the undamaged crista.



FIG. 4 Apposition of an  $\text{NaCl}$  crystal in the round window niche. (On the right side—up—the stapes and the round window.)

FIGS. 5 and 6 See colour plate between pp. 364–5

FIG. 7 The  $\text{NaCl}$  crystal completely dissolved. Perilymph is overflowing from the round window niche to the promontorium and the middle ear.

4 Overflow of the perilymph from the round window fossa in to the bulla and the subtympenic cavity of the middle ear after 4 to 5 minutes (Fig. 7)

#### 5 Stopping of the perilymph overflow

*As to the Deposition of a Crystal of Methylene Blue we observed*

1 Appearance on the window of a dark blue liquid after nearly 1 minute, that is, the outflowing perilymph set free in the methylene blue which is so-coloured.

2 Disappearance of the crystal in 7 to 8 minutes.



FIG. 3. Round window membrane (cat) after exposure of NaCl crystal for 5 minutes.

FIG. 4. Normal round window membrane (cat) (control)

FIG. 5. Collapsed endolymphatic duct (1st phase of the experiment). Irritation: endolymphatic signs; The pulse appears compressed on the undamaged cristae.



3. Overflow of the dark blue liquid from the round window fossa after 18 to 19 minutes.

4. Stopping of the perilymph overflow

In order to verify the structural modifications, the electrophysiological phenomena, and the biochemical and osmotic changes which, in our experiment, have been provoked after the loss of water of the perilymph through the round window membrane to the middle ear cavity we carried out a series of different investigations.

#### *A. Histological Modifications*

The labyrinths of animals sacrificed at different times after the introduction of a saturated saline solution into the middle ear were studied on sections prepared after the modified Wittmaack's technique, both in the injected and in the non injected side (control series)

1. During the first "irritative" vestibular phase we observed

Important modifications at the level of the cochlear canal of the injected side: the findings were very similar to histological pictures found at post mortem examinations of the labyrinths of Ménière's patients, as described by many A.A.

The perilymphatic space appeared extremely dilated and the endolymphatic canal was compressed and collapsed: the tunnel of the organ of Corti remained visible even if the tectorial membrane adhered to the hair cells. In some preparations an homogenization of the protoplasm, both of hair cells and supporting cells, has been observed, so that in some cases it resulted difficult the identification of their cellular contours. The nuclei, however, appeared unaffected (Fig. 8)

The same alterations appeared in the vestibular apparatus (Fig. 9)

2. During the second "paralytic" vestibular phase we observed

The cochlear canal appeared dilated and, while the tunnel of the organ of Corti appeared everywhere compressed, the hair cells and supporting cells showed only modest lesions (Fig. 10)

#### *B. Cochlear Microphonic Variations*

In other series of cats we carried out investigations on the modifications of cochlear microphonics occurring during the different times of the experiment.

The recording electrodes of enameled silver wire, 150 microns in diameter were insulated up to the round window membrane and fixed to the edge of the bulla with dental cement.

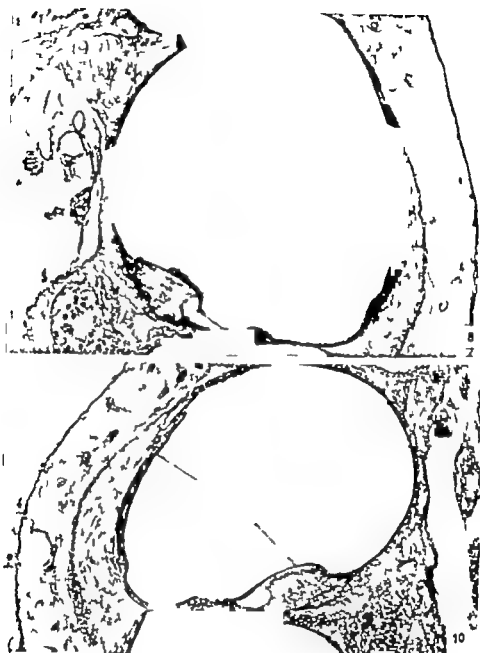


FIG. 8 Collapsed cochlea and dilatation of the perilymphatic space (the organ of Corti appears undamaged) (1st phase of the experiment).

FIG. 9 Section of the cochlea between pp. 364-5.

FIG. 10 Bulging of the endolymphatic duct; the tectal membrane is compressing the undamaged organ of Corti.

The indifferent electrode of silver wire was fitted in the muscles of the neck. Sound stimuli of 250 1000 2000 4000 and 8000 cps (Peter's audiometer mod SPD 5) were given.

We fixed a speculum in the external auditory meatus of the animal and connected it to a loudspeaker through a rubber tube of 100 cm length.

The cochlear microphonic (CM) potentials from the round window were

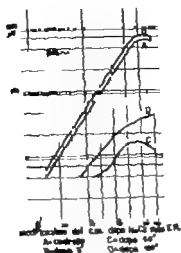


Fig. 11. Modifications of the cochlear microphonics (after apposition of an  $\text{NaCl}$  crystal on the round window membrane (control) — control, dopo 5' — after 5' dopo 60' 120' — after 60' 120').

amplified with an A.C. preamplifier (Tektronix, mod. 122) and were recorded with an oscillograph (E.T.C., mod. 521 A). The C.M. were recorded before having put a small crystal on the round window membrane and after having observed the outflow of the perilymph.

We can summarize our observations as follows:

1. Increase in C.M. immediately after the placement of a crystal of  $\text{NaCl}$  on the round window. This process ended after 1 to 2 minutes, in which time the crystal was completely dissolved.

2. After the first phase, the C.M. potentials reduced gradually reaching the maximum decrease 8 to 15 minutes after the apposition of the  $\text{NaCl}$  crystal on the round window membrane. This reduction of the C.M. potentials involved both the high and the low frequencies.

3. In the second phase the C.M. amplitude gradually increased, but it still remained below the initial values. This takes a longer time than that of the reduction of the C.M. amplitude.

4. When the C.M. has reached a certain amplitude, it falls again.

5. Finally we observed a recovery of all the frequencies, initially more rapid for the high frequencies. In other words, we observed that the low frequencies are more affected than the high ones. This is very probably due to an impairment of the mechanical transmission systems of the cochlea to stiffness increase, as in the first stages of Menière's disease (Fig. 11).

At a certain moment, at the end of the instillation, the amplitude almost reached the initial values for the high as well as for the low frequencies.

The phenomena we observed lasted from 30 minutes for small  $\text{NaCl}$  crystals to 3 hours for the larger ones. The curve of these phenomena

showed that the high frequencies were more intensely involved than the lower ones. It was also characterized by a progressive fall in the amplitude of C.M.<sup>1</sup>

### *C. Action Potential Variations in a Single Delter's Unit*

A further electrophysiological investigation was devoted to the recording of modifications of action potentials registered at the level of the second vestibular neuron during the course of the experiments.

A tracheal cannula was placed in cats and precollicular decerebration performed. Carotid arteries were occluded for 15 minutes. Microelectrodes with steel needles, sharpened electrolytically to tips measuring 10-20 micron in diameter were used. Recording placement was made with a stereotaxic instrument, using a micromanipulator permitting very slow penetration. Electrical activity of single Delterian units was amplified by a preamplifier (Tektronix, mod. 122) and measured by an electronic oscillograph (ETC, mod. 21 A). Bromide paper was used for recording.

After having recorded the responses of the Delterian units to cathodal and anodal polarization of the labyrinth, a small NaCl crystal was placed on the round window membrane.

After the apposition of the crystal on the round window membrane of the ipsilateral middle ear a single Delterian unit responding with an increased frequency to cathodic polarization (and with reduced frequency to anodic polarization) of the ipsilateral labyrinth, presented the following variations:

1. 1 to 2 minutes after the apposition of the crystal, the spontaneous rhythmic discharges increased gradually in frequency and 10 minutes later this increase reached a value three times greater than the initial one. Simultaneously the extension tonus of the ipsilateral limbs increased as well as the amplitude and the frequency of the potentials from the ipsilateral triceps. We observed also a tonic deviation of the eyes towards the opposite side, together with ocular nystagmus.

2. 15-20 minutes later the rhythm became irregular and discharges began to decrease in frequency.

The rigidity phenomena of decerebrated animals at the level of the ipsilateral forelimbs, and the frequency and amplitude of the electromyogram, were reduced, with irregular groups of discharges. The tonic deviations of the eyes were not constant and difficult to observe.

3. After 20 to 30 minutes, the electrical activity of the Delterian units dropped. The extensor tonus of the ipsilateral limbs was remarkably reduced and the electromyogram showed no electrical activity of the muscle. The eyes were deviated ipsilaterally to the ear under experiment, with occasional nystagmus.

4. After this period lasting from 20 to 30 minutes, we observed a slow

This investigation was performed by E. Molinari, M.D.



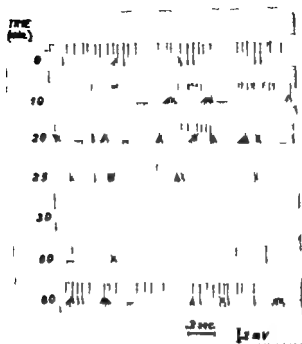


FIG. 12. Modifications of the electrophysiological activity of single unit of Deiters nucleus during the experiment. 0: before the experiment began (spontaneous activity of the unit); 10: 10' after NaCl crystal position (increasing activity (1st phase: excitatory irritability (gas)); 20: 20' after position, etc. decrease of the preceding discharge ratio; 25: 30' after position, etc. disappearance of activity (2nd phase "paralytic" excitability); 60: 60' after position, etc. reappearance of initial activity; 80: 80' after position reappearance of spontaneous activity as at 0.

recovery of electrical activity of the vestibular nuclei. During this period the electromyogram of the ipsilateral triceps returned to the initial values, while the eyes returned to the normal position. After 80 minutes, the frequency of discharges presented the same values recorded before the investigation (Fig. 12).

#### D Ions and Osmotic Pressure Variations

Cats were used for our experiments, allowing an easier exposition of the round window membrane. A sodium chloride crystal was placed on this membrane. After three minutes this crystal was completely dissolved by the liquid filling the inner ear. The whole surface of the middle ear was thoroughly washed and dried. Samples of the scala tympani perilymph were collected by piercing the membrane with a dry glass micropipet carried on a micromanipulator. Endolymph from the scala media was collected with a small micropipet, after removal of the round window membrane, by piercing the basilar membrane in the basal turn.

This investigation was performed by G. M. Linari, M.D.

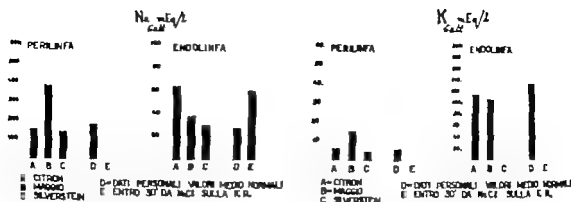


FIG. 13. Cont. nt of Na and K ions in the perilymph and endolymph. A B C, data after Citron, Maggio, and Silverstein D personal data. Valori medionormali=middle values entro 30' da NaCl sulla F.R.=values obtained in the first 30' after apposition of a NaCl crystal on the round window

The microchemical study of sodium and potassium concentration of endolabyrinthine fluids was performed by spectrophotometry with atomic absorption.

Mean values of  $\text{Na}^+$  and  $\text{K}^+$  ions, both in the normal perilymph and in the endolymph of the cat (see Fig 13) were quite similar to those referred to by Citron and Silverstein

Samples collected within half an hour after sodium chloride crystal apposition on the round window membrane showed a very high increase of sodium ions concentration in the perilymph and a less pronounced increase in the endolymph (Fig 13)

As what concerns the mean values of K ions in the perilymph and endolymph and their modifications after sodium chloride deposition on the round window we observed that the increase of concentration was very high in the perilymph but in the endolymph it was very small (Fig 13)

In Fig 14 we have represented the ratio between endo- and perilymphatic sodium and potassium ions

We observed that the value of the sodium ratio was not modified by

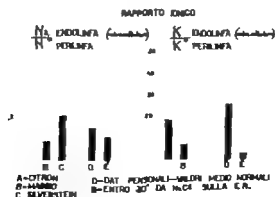


FIG. 14. Ionic ratio of Na and K between endolymph and perilymph (translations of Fig 13)

TABLE 1 Osmotic pressure relative to Na and K ions.

$\pi = \pi' \lambda + \pi'' \lambda$   $\pi = g \cdot T$   
 $\pi$  Osmotic pressure,  $g$ —coefficient,  $\lambda$ —concentration  $T$ —absolute temperature.

	Thor	% NaCl
Normal ionic concentrations		
Perilymph	3044	1.016
Endolymph	2503	0.960
Ionic concentrations after 30' NaCl on the round window		
Perilymph	4445	3.053
Endolymph	3646	1.253
* Increase of the osmotic pressure		
Perilymph		191
Endolymph		45

sodium chloride on the round window whereas the potassium ratio was profoundly modified, varying from a normal value of 25 to a value of 3.

Variations of osmotic pressure were measured in the endolymph and perilymph related to sodium and potassium ions concentrations. Normal values were quite similar to those reported by Aldred *et al* (1940).

After sodium chloride deposition on the round window osmotic pressure increased by 191% in the perilymph and 45% in the endolymph (Table 1).

These results call attention to three principal phenomena:

1. Global increase of the ionic concentration and consequently of the osmotic pressure.

This is connected to a water subtraction from endolabyrinthine liquids. This water through the round window membrane, passes from a less concentrated solution to a more concentrated solution produced by the sodium chloride crystal.

2. Higher concentration of sodium in the perilymph than in the endolymph. It is possible to explain this phenomenon by admitting that a small quantity of sodium chloride placed on the round window passes into the perilymph of the scala tympani.

3. Increase of the potassium concentration in the perilymph, while in the endolymph the concentration is near to normal.

It is possible to explain this phenomenon by admitting a potassium passage from endolymph to perilymph. In other words, the increase of potassium concentration in the endolymph, resulting from water subtraction, is cancelled by subsequent passage of potassium into the perilymph. Table 1 summarizes hypothetically the succession of phenomena in the 1st phase of the experiment.

These investigations were carried out by G. Perin, Ph.D. and G. Mollnert, M.D.

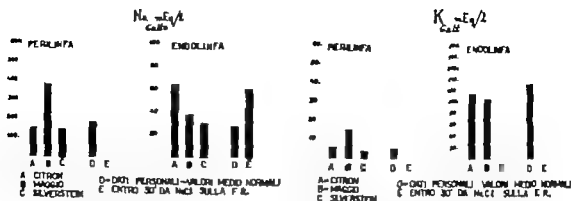


FIG. 12. Content of Na and K ions in the perilymph and endolymph. A B C, data after Citron, Maggio, and Silverstein. D personal data. Valori medio normali = middle values entro 30' da NaCl sulla F.R. = values obtained in the first 30' after apposition of a NaCl crystal on the round window.

The microchemical study of sodium and potassium concentration of endolabyrinthine fluids was performed by spectrophotometry with atomic absorption.

Mean values of Na and K ions both in the normal perilymph and in the endolymph of the cat (see Fig. 13) were quite similar to those referred to by Citron and Silverstein.

Samples collected within half an hour after sodium chloride crystal apposition on the round window membrane showed a very high increase of sodium ions concentration in the perilymph and a less pronounced increase in the endolymph (Fig. 13).

As what concerns the mean values of K<sup>+</sup> ions in the perilymph and endolymph and their modifications after sodium chloride deposition on the round window we observed that the increase of concentration was very high in the perilymph but in the endolymph it was very small (Fig. 13).

In Fig. 14 we have represented the ratio between endo- and perilymphatic sodium and potassium ions.

We observed that the value of the sodium ratio was not modified by

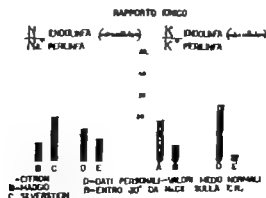


FIG. 14. Ionic ratio of Na and K between endolymph and perilymph (the same as in Fig. 13).

TABLE 1 Osmotic pressure relative to Na and K ions

$\pi = \pi_{Na} + \pi_K \quad \pi = g \quad T$   
 $\pi$  Osmotic pressure,  $g$ —coefficient,  $c$ —concentration,  $T$ —absolute temperature.

	Theor	% NaCl
Normal ionic concentrations		
Perilymph	3044	1.018
Endolymph	2503	0.960
Ionic concentrations after 30' NaCl on the round window		
Perilymph	3325	3.053
Endolymph	3646	1.253
% Increase of the osmotic pressure		
Perilymph		191
Endolymph		45

sodium chloride on the round window; whereas the potassium ratio was profoundly modified, varying from a normal value of 25 to a value of 3.

Variations of osmotic pressure were measured in the endolymph and perilymph related to sodium and potassium ions concentrations. Normal values were quite similar to those reported by Aldred *et al.* (1940).

After sodium chloride deposition on the round window osmotic pressure increased by 191% in the perilymph and 45% in the endolymph (Table 1).

These results call attention to three principal phenomena:

1. Global increase of the ionic concentration and consequently of the osmotic pressure.

This is connected to a water subtraction from endolabyrinthic liquids. This water through the round window membrane, passes from a less concentrated solution to a more concentrated solution produced by the sodium chloride crystal.

2. Higher concentration of sodium in the perilymph than in the endolymph. It is possible to explain this phenomenon by admitting that a small quantity of sodium chloride placed on the round window passes into the perilymph of the scala tympani.

3. Increase of the potassium concentration in the perilymph, while in the endolymph the concentration is near to normal.

It is possible to explain this phenomenon by admitting a potassium passage from endolymph to perilymph. In other words, the increase of potassium concentration in the endolymph, resulting from water subtraction, is cancelled by subsequent passage of potassium into the perilymph. Table 2 summarizes hypothetically the succession of phenomena in the 1st phase of the experiment.

These investigations were carried out by G. Perin, Ph.D., and G. Molinari, M.D.

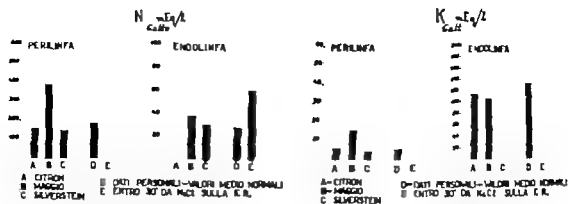


FIG. 13. Cont. of  $\text{Na}^+$  and  $\text{K}^+$  ions in the perilymph and endolymph. *A B C*, data after Citron, Maggio, and Silverstein. *D* personal data. *Valori medianormali*—middle values entro 30' da  $\text{NaCl}$  sulla PR—values obtained in the first 30' after apposition of a  $\text{NaCl}$  crystal on the round window.

The microchemical study of sodium and potassium concentration of endolabyrinthine fluids was performed by spectrophotometry with atomic absorption.

Mean values of  $\text{Na}^+$  and  $\text{K}^+$  ions, both in the normal perilymph and in the endolymph of the cat (see Fig. 13) were quite similar to those referred to by Citron and Silverstein.

Samples collected within half an hour after sodium chloride crystal apposition on the round window membrane showed a very high increase of sodium ions concentration in the perilymph and a less pronounced increase in the endolymph (Fig. 13).

As what concerns the mean values of  $\text{K}^+$  ions in the perilymph and endolymph and their modifications after sodium chloride deposition on the round window we observed that the increase of concentration was very high in the perilymph, but in the endolymph it was very small (Fig. 13).

In Fig. 14 we have represented the ratio between endo- and perilymphatic sodium and potassium ions.

We observed that the value of the sodium ratio was not modified by

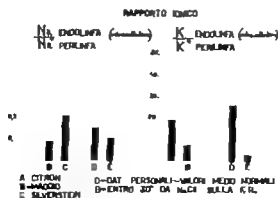


FIG. 14. Ion ratio of  $\text{Na}^+$  and  $\text{K}^+$  between endolymph and perilymph (translation in Fig. 13).

of water ultrafiltration (increase and decrease of the reabsorption of the perilymph and the endolymph) appears fully justified if it corresponds to as many stages of variations of the osmotic balance between the endolymph and the perilymph.

As a matter of fact, concerning Reissner's membrane, the limbus spiralis, and the other structures, recent investigations with electronmicroscopy (Spoendlin, 1964; Jurato, 1967; v. Ilberg, 1968, and others) seem to give confirmation to their permeability.

For example, Jurato (1967) states: "from a morphological point of view that it can be stressed that the extreme thinness of the membrane of Reissner certainly permits diffusion phenomena. Moreover the pinocytotic vesicles demonstrated both in the epithelial and in the connective cells, could represent some morphological evidence for the passage of fluids through the membrane of Reissner" (page 80).

As in our experiments biochemical factors are neither introduced in the labyrinth cavities nor in the vascular system (blood, lymph, liquor) it appears evident that only biophysical-induced variations in the labyrinth fluids occur and that they are cause of the observed phenomena.

In fact, we must admit that the regulation of the perilymphatic and endolymphatic function is not only based on biochemical exchanges, but even on biophysical parameters, as osmotic pressure, hydrostatic energy, weight, etc. Between the two liquids there is undoubtedly not only a biochemical exchange of ions, molecules, etc., but also some biophysical mechanism which ensures the reciprocal balance of osmotic pressure, of hydrostatic energy, etc. The permeability of the labyrinth membranes and vessels is certainly an extremely important dynamic biophysical regulating mechanism. Actually an alteration of this permeability is followed by a modification of the normal ratio between the ions inside and outside sensory receptors, as we pointed out. The alteration of this ratio provokes a dramatic change in the cochlear resting potentials, as the Nernst's equation shows (Molinari, in press).

#### *Analogies of Experimental Findings and Pathogenesis of Menière's Attacks*

A very important point of discussion appears to be the striking similarity existing between the course of these experiments and the clinical picture of the vertigo attack in Menière's disease. In fact, if we analyze the pattern and temporal evolution of the Menière's attacks, in their typical features, we find finally the presence of "irritative" vestibular signs (nystagmus beating to the ill side, etc.) "paralytic" signs can be observed too, but with a smaller frequency. Furthermore when the attack is ending, practically only the "paralytic" signs are to be seen.

Moreover the Lermieux phenomenon (increase of the tinnitus, and sometimes of the hearing, before the dizziness attack) acquires the meaning of something which transiently "stimulates" the inner ear.

TABLE 2 *Phenomena occurring in the first phase of the experiment after NaCl on the round window*

1 Increase in the osmotic pressure of the labyrinthine fluids, which produces	
2 Alteration in the permeability of Reissner's membrane followed by	
3 Decrease of the ratio between the inner and outer $K^+$ concentration, which causes	
4. Decrease of resting potentials of the labyrinthine receptors, which provokes	
Cochlear partition	Vestibular partition
1 Decrease of CM which is responsible together for the increase of osmotic pressure of	1 Increase in the spontaneous activity of the vestibular nerve, which is responsible for
2. Typical deafness due to involvement of transmission system of the cochlea	2. Typical attacks of vertigo irritative nystagmus, etc,

### COMMENT

Osmotic balance between the labyrinthine fluids is certainly regulated by the following mechanisms

(1) Secretion and absorption of the fluids with alternative dynamic processes and (2) passage of water ions, and molecules, especially through the Reissner's membrane, ligamentum spirale, prominentia spiralis, vasculo-epithelial zone of Borghesan and other structures (v Ilberg 1968, and others AA)

Concerning the stria vascularis, it performs a double function both of secretion and absorption—this seems to be clearly shown by the electron microscopic investigations of Spoendlin (1964) Reinecke (1968) and others AA)

On the other hand, Rauch (1966) has demonstrated that the whole labyrinthic liquid system (endolymph and perilymph) having a high dynamic metabolism is totally changed in a radial direction nearly every 10 minutes.

The hypothesis that a mechanism of water ultrafiltration, besides a dislocation of ions and proteins, take place in the vessels of the vascular stria and of other secreting structures of the labyrinth, is therefore justified. This process of water ultrafiltration resembles the equal process taking place in the renal tubulus, where the glomerulus secretes nearly all the water and this water is reabsorbed by particular cells soon after reaching the tubulus. Then the alternate mechanism of secretion and absorption of the labyrinth fluids could take place also in the labyrinth vessels, since the smallest experimental withdrawal of water from the perilymph causes—as we have seen—important functional variations, both histologic and electrophysiological.

As our findings show that the artificial variation of the osmotic pressure of the perilymph provokes functional alterations, first in one direction (irritative syndrome) and then in the contrary one ("paralytic" syndrome) the hypothesis of some compensative mechanisms (sudden increase



Des grandes analogies apparaissent entre l'expériment et les crises aiguës du Menière en fait, dans cette crises, les signes irritatifs précèdent ceux paralytiques. En outre, le collapsus du canal endolymphatique qui a été observé dans le Menière initial et la 1<sup>re</sup> phase de l'expériment, sont les mêmes.

## ZUSAMMENFASSUNG

Die Einführung einer gesättigten NaCl-Lösung in das Mittelohr des Meerschweinchens oder der Katze ruft ein spontanes biphasisches Vestibularsyndrom hervor gebildet aus 1 Phase „irritativer“ Nystagmus usw 2. Phase paralytischer Nystagmus usw Der Verf. hat bewiesen, dass dieses Experiment histologische, elektrophysiologische, biochemische, biophysische sehr interessante Veränderungen hervorruft, deren Hauptursache der osmotische Druck zu sein scheint.

Es scheinen grosse Analogien zwischen dem Experiment und den akuten Menierekrise zu bestehen.

## REFERENCES

- ALLEN, P. HALLPINE, C. S., and LINDOY, A., 1940 Observations on the osmotic pressure of the endolymph. *J Physiol* 92, 339.
- ALTMAN, F. and WALTHER, J. G. 1930: Further investigation of the physiology of the labyrinthine fluids. *Ann. Otol.* 39 637.
- ARMAS, M., 1944: Experimental Menière's disease. From "The vestibular system and its diseases" Edited by W. H. H. H. Philadelphia, University of Pennsylvania Press.
- ARMAS, M., BONICERAT, E., JEMATO, S., MARINO, F., DEL BO' CASIO, S., and MONTANA, G. 1944 Symposium sull'etiopatogenesi della malattia di Ménière. Verona, 27 sett. in press.
- ARMAS, M., BALI, O., GACCONELLI, F. and MONTANA, G., 1943 International Symposium on Menière disease. *Suppl. Arch. Ital. Otol. Rhinol. Laringol.*
- ARMAS, M., 1943 Auskleidung des Perilymphraumes im Innenohr. *Z. Rhinol. Otol.* 5, 47.
- JEMATO, S. *et al.*, 1947 Submicroscopic structure of the inner ear Pergamon Press, London.
- KIRITA, R. S., 1947 Experimental blockage of the endolymphatic duct and its effect on the inner ear of the guinea pig. (A study on endolymphatic hydrops.) *Ann. Otol.* 66.
- MONTANA, G. In press.
- NAPHTALIN, L. and HARRISON, M. S., 1944 Biochemistry of the labyrinthine fluids. From "The vestibular system and its diseases" by R. W. H. H. Philadelphia, University of Pennsylvania Press.
- PERIN, G. and MORELLI, G. U. metodo per la titolazione degli ioni sodio e potassio nella perillina dell'endolinfa del gatto. In press.
- RECH, S., 1944 La liasse d'échange des liquides labyrinthiques. Comptes rendus du 63 Congrès Français d'ORL, Paris.
- 1947 New morphological and functional results on the vascular system of the inner ear. *Otolaryng. Folia*, 13.
- SCHROEDER, H. F., GUNTER, W. L., DAVIS, G., and SALVENDY, H., 1943 Chemical evaluation of inner ear fluids as diagnostic aid. *Acta Otolaryng.* (Stockh.) 65, 149.

The fluctuating hearing which is a typical cochlear sign of Menière's disease, clearly indicates, too, that the pathogenic processes of this disease are changing moment by moment in their evolution.

The parallelism we point out between our experimental data and the patterns of the clinical pictures of Menière's disease means that Menière's attacks perhaps depend on quick alterations of the osmotic balance between the labyrinthine fluids.

As a matter of fact, recent biochemical investigations on the inner ear fluids of Menière's patients have shown that there is no variations from normal values (in the uncontaminated samples of perilymph and endolymph removed from the vestibule of these patients) concerning sodium, potassium, and protein content (Schuknecht 1968, Rauch 1966, and others). This fact seems to mean that Menière's attacks do not depend directly on ionic changes of the labyrinth fluids. On the other hand, the hydropic lesions, which are the constant pathologic finding in this disease, are to be considered only as the final phase of long chain of primary pathogenic factors whose beginning occurred many years before the first attacks. These first pathogenic factors of Menière's disease take place mainly in the delicate ultrafiltration mechanism of water in the labyrinth vessels: ultrafiltration is strictly dependent on the permeability mechanism, on the biochemical blood content and so on. But the primary pathology in the disease can be, from a clinical point of view, silent as the regulation between the perilymph and the endolymph of water ultrafiltration of reciprocal biochemical exchanges, of osmotic balance, etc., can be quickly compensated and return to normality. Afterwards, the progressive impairment of the vessels pathology reaches, at a certain moment, a loss of the biochemical and osmotic balance: at this moment, the first Menière's attack appears in all its clinical patency.

The first phase of the experimentally induced loss of osmotic balance corresponds, as we have seen, to a collapse of the endolymphatic duct: there are in the literature many findings of this lesion in human Menière's pathology and the "collapse" precedes the formation of bulging lesions of later occurrence.

## RÉSUMÉ

L'introduction d'une solution saturée de Chlorure de sodium dans l'oreille moyenne de la cobaye ou du chat, déclenche un syndrome vestibulaire à pontanée biphasique composée par : 1<sup>re</sup> phase des signes irritatifs (nystagmus battant vers l'oreille injectée, etc.) 2<sup>de</sup> phase des signes paralytiques (nystagmus battant vers le côté non injecté, etc.)

L.A. et ses collaborateurs ont démontré que cet experiment cause des variations histologiques, électrophysiologiques, biochimiques, biophysiques (variations de la pression osmotique des liquides labyrinthiques) très intéressantes. La pression osmotique paraît être la cause principale des variations obtenues expérimentellement.

does not support the view depicted by Professor Aralan's histologic specimen after the drastic changes due to sodium chloride application. Transient depolarizations by potassium from the endolymph, on the other hand, doesn't produce any histologic changes. A slight bulging of Reissner's membrane in no way of the cochlea and a sinking in another is a common artefact which has nothing to do with the distortion of the whole endolymphatic tube which Kimura has produced by blocking the endolymphatic duct and sac. His results seem to show hydrop of the labyrinth comparable to that in Ménière cases. As far as I remember Kimura has also told me that these animals occasionally have attacks of vertigo comparable to the Ménière attacks. The changes in action potential frequency shown by Professor Aralan are in accordance with what must be expected from vestibular stimulation regardless of the mechanism by which it is stimulated.

Mr Aralan (Réponse) à Mr Portmann 1ère question Les microphoniques cochléaires pendant la période paralytique rentrent dans la normalité surtout dans les fréquences aigües. Ceci est l'indice d'une amélioration fonctionnelle.

2ème question Je n'ai pas fait des expériences ouvrant le sac endolymphatique.

To Mr Friedmann I did not carry out electromicroscopic controls in our experiments. But we have planned it.

à Mr Montandon Vous n'avez pas fait des examens E. V. G. dans nos expériences. Vous les ferez.

T Mr Spondlis The suggestion is right and we will immediately begin the histological researches on the modifications which can appear on the round window membrane after apposition of NaCl crystal. It is quite possible that the coming out of perilymph water in the middle ear depends from the histological lesions of round window membrane but we payed more attention to the functional effects of the experiences and to the electrophysiological, biochemical, osmotic variations, than to the local mechanisms of the outcoming of perilymph.

T M Dohmann I agree that it is not possible to identify our experiments with Ménière attacks. But we believe hypothetically that by the two different condition (the functional biphasic vestibular spontaneous signs) which are the same by Ménière's disease as by the experiments, depend in both from acute osmotic changes between perilymph and endolymph. On the other hand we never saw your histological preparations a rupture of Reissner's membrane. I agree that the vestibular cells and the Corti cells remain normal by acute collapse or successively by acute hydrops. I also agree that the hydrop obtained in Kimura's experiment is picture whose mechanism is completely different from that we saw in our experiment. In fact, we have never seen a typical hydrop of cochlear duct, only "bulging" of Reissner's membrane and a compression of the Corti organ, which appears only in the second phase of the vestibular syndrome ("paralytic") produced by the experiment. We are sure that the histological pictures we observe are not artefacts as we compared them with the histology of the opposite side of experimented animals. I agree that in both situations (Kimura's experiments and mine) functional Ménière-like signs can be observed, but the fact that of ion content doesn't change by Ménière patients (Schuknecht, and other AA.) is a valuable argument in favour of osmotic pathogenesis of Ménière's attack, as we demonstrated by our experiments.

- SPORNBLIX, H. H. 1964: Ultrastructure of the vestibular sense organ. From "The vestibular system and its disease" by R. Wolfson, Philadelphia, University of Pennsylvania Press
- TOMNORBY, J. 1967: The mechanism of hearing loss in early cases of endolymphatic hydrops. *Ann. Otol.* 66: 766

*ENT Department  
University of Padua,  
Padua, Italy*

## DISCUSSION

*A. Laskiewicz* I would like to mention that Mr Lindsay and Mr Kimura (Chicago) in 1951 evoked experimentally in animals Ménière-like symptoms by destroying the sacculus endolymphaticus or tightening the ductus endolymphaticus (Kimura) in guinea pigs and rabbits. May I suggest a comparison between the method of Rossem & Arslan with that of the above-mentioned authors, whose experiments have proved in animals the dilatation of the whole endolymphatic system.

*M. Portmann* Très claire démonstration. Seulement 2 questions: (1) Mr Arslan montre une augmentation du phénomène microphonique pendant la période paralytique. Est-ce dans son esprit le signe d'une hyperfonction au point de vue auditif? (2) A-t-il fait la même expérience après l'ouverture du sac endolymphatique ou après intercommunication entre perilymphe et endolymphé?

*J. Friedmann* I wonder whether certain electronmicroscopic studies on his experimental material might assist in confirming our EM-findings, e.g. the presence of intracellular crystalline inclusions and broad-banded collagen.

*A. Montandon* L'expérience d'Arslan qui n'a pas la prétention de démontrer le mécanisme exact de la crise de Ménière mais seulement d'en évoquer l'une des possibilités, a le grand mérite de faire intervenir l'élément vertigineux, ce que n'est pas le cas en général dans l'hydrops expérimental. J'aimerais lui demander si en plus de la dérivation des potentiels des fibres nerveuses vestibulaires, il a utilisé l'enregistrement ENG qui se rapproche des tests cliniques usuels?

*H. Spoendlin* I would like to ask Professor Arslan whether he has examined histologically the round window membrane during such experiments. I wonder whether only osmotic phenomena play a role in these experiments. We did similar studies in the cat and we found the same labyrinth reaction when we introduced a saturated solution of NaCl but no reaction when we introduced other hypertonic solutions such as solution of saccharose and mannitol.

*G. Dahlman* The experiments which Professor Arslan described are certainly suited for demonstrations to students of vestibular reactions, but I can't see what connection these experiments might have with Ménière's disease. As far as I know there has never been shown any evidence of such spectacular osmotic changes or perilymphatic sodium increase in Ménière cases. On the other hand the ruptures in the walls of the distended vestibula sacs which Professor Arslan mentioned seems to me more profitable for a closer study. As is well known, the potassium of the endolymph has a concentration sufficiently high to produce a depolarization of the vestibular nerve fibres, as I earlier have been able to show. The intact vestibular hair cells even in Ménière cases of long duration

## VESTIBULAR NEURONITIS

M. SPENCER HARRISON

*From the National Hospitals for Nervous Diseases London, England*

A number of syndromes have been described which are similar to vestibular neuronitis, all with vertigo as their chief symptom, in some the vestibular signs and symptoms have occurred alone, in some series evidence of epidemic nature was noted, in others neurological changes such as affection of the oculomotor nerve. By a follow-up of 60 cases of vestibular neuronitis it is shown to be a separate disease entity in which a certain diagnosis can be made. Statistics of age and sex incidence, of the number of attacks and their duration and severity are tabulated. A severe vestibular upset leads frequently to habituation of one or both vestibular systems to stimuli producing an absence of caloric responses in an otherwise normal system. A similar form of habituation occurs in dancers and ice skaters and is associated with certain employments, thus when the caloric responses are depressed or absent a history covering these possible causes of habituation should be taken.

The condition, now well-known, which was called vestibular neuronitis by Hallpike (1949) cannot be distinguished from Menière's disease by the nature of the vertigo, but solely by the absence of cochlear signs and symptoms. It affects younger adults than does Menière's disease. In a few cases the onset is associated with a febrile illness or an infection in the nose or throat, but as in Menière's disease, infective ear disease does not seem to be a responsible factor. The disorder may complicate conditions such as brucellosis (Thompson & Bernstein, 1963). Tinnitus or deafness are notably absent and the most detailed tests of cochlear function give normal results, but tests of vestibular function are invariably abnormal. Positional nystagmus, type II (benign paroxysmal) (Dix & Hallpike, 1952) or at times type III (Harrison, 1966) is often found in vestibular neuronitis and in other severe lesions of the vestibular system such as Menière's disease in which the vestibular "tonus" is temporarily lost during changes of position (Hart, 1963).

In most cases the caloric tests show severe derangement. The degree of disturbance of response depends largely upon the acuteness of the affection and the time between the onset and the test. In only a few cases does a unilaterally depressed vestibular response persist and when it does, the lesion is almost always a severe one. The difference between the severe lesion which continues to produce a marked depression of the caloric responses and the mild lesion is so remarkable that the two may be different conditions. In the latter the caloric responses usually show only an abnor-



unsteadiness might last several weeks, and especially noticeable on movement, particularly stooping. Following this, 50 personal cases were described by Worster Drought (1953) in some nystagmus was observed. This lasted for several days, but sometimes recurred in a lesser degree during subsequent attacks of vertigo, symptoms resolved slowly usually in 3 to 4 weeks and rarely persisted 12 months. Even with rapid recovery patients were often very unsteady for some time. Walford (1952) suggested influenza as a cause of this type of labyrinthitis and cited an "epidemic" of four cases in which there had been contact with cases of clinical influenza in the same household though examination of the blood showed no evidence of virus infection. A similar series of cases occurred in the U.S.A. (Merrield, 1955).

A large series of cases was reported in which upper respiratory infection was followed by prolonged vertigo (Herberts, 1954). The neurological examination was normal and tests of peripheral vestibular function were normal too. The condition was attributed to a virus encephalitis, a view supported by the finding of an increase of cells and protein in the cerebro-spinal fluid.

Dalsgaard-Nielsen (1953) also described "epidemic vertigo" in which electroencephalographic abnormalities were found in 18 out of 21 of the cases with some correlation with the clinical course of the condition. One case complicated by hepatitis and another by epidemic hiccough, often seen in encephalitis lethargica (Economo, 1929) had normal electroencephalograms. All of Dalsgaard-Nielsen's subjects had been in contact with other patients with vertigo and he therefore attributed the condition to an acute cerebral disturbance, probably a virus encephalitis. Nineteen per cent showed hearing loss, though further details of the otolaryngological findings were not reported, but neurological changes were present in 24 per cent, including diplopia in 10 per cent with reflex and sensory changes in others. Twenty-four per cent showed increased sedimentation rates. The cerebro-spinal fluid was normal in the 18 cases in which it was examined.

A similar series of 100 cases of a syndrome closely resembling that of Dalsgaard-Nielsen was reported (Winther) in 1952. The caloric tests were normal in 41 of the 38 patients tested and there were mild changes in the cerebro-spinal fluid in nine out of the remaining 32 cases. The pathological process appeared to be a widespread one thus throughout the central nervous system with vestibular symptoms as the predominant and sometimes only symptoms.

The acute condition  
13 hours to

The larger  
in a series of  
postero-lateral  
months after  
a complaint

Winther (1954) closely resembles the  
slower taking up to  
weeks is greater  
described 400 cases  
a types. Type 1—  
for an interval of  
patients there was  
The vertigo was

mal pattern which soon returns to normal Vestibular palsy of sudden onset occurring as a single attack only has been described (Mehmke, 1963) as a separate disease entity A few of these cases may be the result of an occlusion of the vessels supplying the vestibular end-organs from the internal auditory artery in the case of spasm the severity might depend upon the duration of occlusion Hallpike (1949) found that bilateral derangement of the vestibular system was often present, but other authors (Stahle, 1966) state that the condition is invariably unilateral

In many cases the condition is a benign one. The evidence of vestibular disease with the absence of cochlear symptoms and signs was thought to localise the lesion to the vestibular pathways at some point central to the labyrinth, but Hallpike could not specify the particular neurones or neuronal elements which were involved so that the name vestibular neuronitis was left open purposefully

#### *Differential diagnosis (Wlodyka, 1967)*

It may be said that although vestibular neuronitis shows some features common to all of the other conditions to which reference will be made in this survey yet the resemblance is no more than superficial Thus, it is distinguishable from the epidemic vertigo of Pedersen by its failure of exhibit any true epidemic characteristics and by the constant finding of abnormalities of the caloric responses

Further the absence in vestibular neuronitis of any neurological abnormalities outside the eighth nerve system clearly separate it from vertigo due to brain stem encephalitis with its associated oculomotor and other neurological disturbances (Poston, 1926 Pedersen, 1959 Leishman 1955) It seems clear that we have to deal with a number of nosological entities These have become loosely grouped within a nomenclature which is both inadequate and confusing, and indeed, the terms epidemic vertigo, epidemic labyrinthitis and vestibular neuronitis are often used for any or all of them with little regard to their distinctive clinical and pathological features Clarification of this situation clearly requires that these features should be more clearly specified.

A number of similar syndromes, all pursuing a benign course with vertigo as their chief symptom, have been described in the literature during the course of the last 40 years Vestibular epidemic encephalitis was described by Barré & Reys in 1921 and by Poston in 1926 In some of the cases described vestibular signs and symptoms occurred alone In others oculomotor disturbances were the only neurological abnormalities, accompanied by variable lethargy Encephalitis was postulated as the cause of the condition but no supporting cerebro-spinal fluid data were given

In the past decade several other syndromes have been described, with vertigo as their leading symptom In 1962 attention was drawn in *The Lancet* to the condition epidemic labyrinthitis In this the vertigo was continuous or paroxysmal gradually disappearing in 2 or 3 days, though mild



TABLE 1 Age incidence of some different forms of vertigo

	0-20	21-30	31-40	41-50	51-60	61-70	+70	Total
Ménière's disease	17	40	85	152	161	85	23	573
Vestibular neuronitis	4	10	34	28	18	11	2	108
Positional nystagmus								53
Type I	1	8	12	28	6			53
Type II	3	18	24	65	74	33	3	230
Type III				2	3	1		6
Acoustic neuromas	4	27	40	50	33	3		157

The figures of acoustic neuromas are taken from C. H. Edwards & J. H. Paterson, *Brain*, 74 part 2, 144 (1951)

### Age and sex

The condition is seen most often in the fourth decade and two-thirds of the cases occur in the age group 30 to 60. Thus the peak appears in a younger age group than Ménière's disease. Table 2 shows that it occurs more frequently in males.

### Electro-nystagmography

Aschan & Stahle (1956, 1967) find that the electronystagmograph plays an important part in the diagnosis of vestibular neuronitis, showing spontaneous or positional nystagmus even in cases where it is not visible to the naked eye and so facilitating interpretation of the caloric test.

### Pathology

Vestibular neuronitis is not a fatal condition so that in no case has the histology been reported, but the findings in a patient with vertigo due to sudden unilateral loss of vestibular function without cochlear symptoms were reported in 1956 (Lindsay & Hemenway). The lesion consisted of degeneration of part of Scarpa's ganglion and of the nerve to the utricle.

TABLE 2 Age and sex incidence of vestibular neuronitis

	0-20	21-30	31-40	41-50	51-60	61+	Total
Males	2		17	31	10	4	64
Females	2	3	17	7	9	9	47
Total	4	10	34	38	19	13	108

attributed to dehydration and intoxication. Six patients showed spontaneous nystagmus. The eighth nerve function was tested in two cases only; the responses were normal in one and there was directional preponderance of the caloric responses in the other. Type II—upper respiratory vertigo was present in all the 50 cases and in seven was characteristic of the benign paroxysmal positional type (Dix & Hallpike, 1952; Harrison, 1956). No mention is made of the results of any detailed tests of eighth nerve function. However, some vestibular tests were carried out in 16 of the severest cases of the two groups who were admitted to hospital and it is noteworthy that although in all of these vertigo was a prominent symptom, yet the tests gave normal results in all but three cases. The disorders described by Pedersen therefore differ from the vestibular neuronitis of Dix & Hallpike in two obvious ways. Firstly, in their epidemic character which does not appear to be a feature of vestibular neuronitis. Secondly, and more important, the infrequency and trifling character of any evidence of organic vestibular derangement as indicated by the results of the caloric tests. In contrast with this, Dix & Hallpike found the caloric responses abnormal in 100 per cent of cases with vestibular neuronitis. In spite of the negative results in his cases of the vestibular tests, Pedersen argued in favour of an organic affection of the vestibular tracts within the brain stem, agreeing with the views of Dix & Hallpike.

Epidemics of vertigo have been described from Melbourne from time to time (Williams, 1963) with rising serum antibody titres to coxsackie virus and an increased number of cells in the cerebro-spinal fluid. The caloric responses in these cases were normal. In Sydney (Basser, 1964) attacks of vertigo in children of short duration occur at intervals of several weeks, at times for years. The caloric responses were depressed and no serological abnormality was evident.

### *Incidence*

Vestibular neuronitis is less common than Menière's disease and positional vertigo. Stahle (1966) found that in the Uppsala Clinic Menière's disease was ten times more common but in Great Britain vestibular neuronitis is twice as frequent as in Sweden (Table 1).

### *Epidemic characteristics*

When vertigo affects several members of a fairly localised community it is not unusual for the family doctor to accept this as evidence of an epidemic. The condition may be associated with oro-nasal infections and as these tend to occur in certain seasons such as winter and spring (Gramowski, 1964) so vestibular neuronitis may at times assume apparently epidemic character though often the so-called epidemic is comprised of entirely unrelated types of vertigo (Harrison, 1962).

TABLE 1 Age incidence of some different forms of vertigo

	0-20	21-30	31-40	41-50	51-60	61-70	+70	Total
Utric- ular								
disease	17	40	95	132	161	85	23	573
Vestibular neuritis	4	10	34	28	19	11	2	108
Positional nystagmus								
Type I	1	8	12	26	6			53
Type II	2	18	34	63	74	83	3	290
Type III				2	3	1		6
Acoustic neuroma	4	27	40	50	33	3		157

The figures of acoustic neuroma are taken from C. H. Edwards & J. H. Paterson, *Brain*, 74 part 2, 144 (1951)

### Age and sex

The condition is seen most often in the fourth decade and two-thirds of the cases occur in the age group 30 to 60. Thus the peak appears in a younger age group than Menière's disease. Table 2 shows that it occurs more frequently in males.

### Electronystagmography

Aschan & Stabile (1956, 1967) find that the electronystagmograph plays an important part in the diagnosis of vestibular neuritis, showing spontaneous or positional nystagmus even in cases where it is not visible to the naked eye and so facilitating interpretation of the caloric test.

### Pathology

Vestibular neuritis is not a fatal condition so that in no case has the histology been reported, but the findings in a patient with vertigo due to sudden unilateral loss of vestibular function without cochlear symptoms were reported in 1936 (Lindsay & Hemenway). The lesion consisted of degeneration of part of Scarpa's ganglion and of the nerve to the utricle,

TABLE 2 Age and sex incidence of vestibular neuritis

	0-20	21-30	31-40	41-50	51-60	61+	Total
Males	3	7	17	21	10	4	62
Females	2	3	17	7	9	9	47
Total	5	10	34	28	19	13	108

TABLE 3 *Numbers of attacks occurring in each of a series of 108 cases of vestibular neuronitis*

1	2	3	4	5	Over 5
55	14	11	6	4	18

the superior and the horizontal semicircular canals on the affected side. A mass of fine convoluted vessels in the internal auditory meatus invading the ganglion of Scarpa indicated vascular occlusion as the cause of the acute process.

The vertigo in this case appears to have shown more positional features than is usual in vestibular neuronitis and Lindsay does not consider that the clinical findings should lead to a diagnosis of vestibular neuronitis.

### *Prognosis*

Vestibular neuronitis is benign in the majority of cases but the duration of the disability after the acute vertigo varies widely from a few days to 1 or 2 years or more and is related in most cases to its severity. After the severe vertigo a period of unsteadiness ensues, with, at times, shorter and less severe attacks of vertigo (Table 3). The unsteadiness gradually becomes little more than an insecure feeling on rapid head movements. It is rare for the unsteadiness to cause prolonged handicap, in these cases the caloric responses remain nearly absent, in many of the rest of the cases little abnormality can be found in the responses to hot and cold caloric stimuli by the end of a year (Table 4).

Table 5 confirms, as would be anticipated, that when the condition is severe it tends more often to be prolonged though 14 per cent of cases with mild unilateral disease are also of considerable duration.

### *Diagnosis*

One of the criteria of diagnosis in the present series has been the absence of cochlear symptoms and signs. Certain peripheral conditions may at times produce only mild cochlear abnormalities (Pfaltz, 1955; Boffi, 1965) but we agree with Hallpike that this should distinguish such lesions from the condition with which we are at present concerned.

From the criteria outlined in the initial paragraphs it is possible to make

TABLE 4 *Duration of vestibular neuronitis in 108 cases*

< 1/52	< 1/12	< 3/12	< 6/12	6/12 +
26	28	21	8	25

TABLE 5 Duration and severity of cases of unilateral and bilateral vestibular neuronitis

Severe			Moderate			Mild		
			Unilateral					
34 cases			21 cases			25 cases		
Short duration	Medium duration	Prolonged	Short duration	Medium duration	Prolonged	Short duration	Medium duration	Prolonged
8	8	10	6	10	5	21	3	4
			Bilateral					
20 cases			1 case			2 cases		
5	2	13	1	—	—	2	—	—

a reasonably accurate diagnosis but much doubt has been expressed on the ultimate outcome and final diagnosis in cases appearing initially to be vestibular neuronitis. Thus 60 patients who had suffered from vestibular neuronitis from 15 years to 5 years previously that is in all of them the initial attack was more than 5 years before, were reviewed in the outpatient department with their unit system notes. Their family doctors were also asked to supply information of other illnesses these patients had contracted since the attacks of vertigo. All these patients derived from a relatively isolated community of approximately half a million persons in two hospital groups. The case notes in the unit note systems of the hospital group contained records of most of the details of these patients' hospital attendances. These three lines of approach—the patient, the family practitioner and the hospital notes—provided a comprehensive history of the patients' illnesses.

In one of the 60 patients the diagnosis had since been changed to Menière's disease and in one other case the diagnosis was considered doubtful as the clinical findings were not clear cut. In none of the others was a change of diagnosis indicated and in none of the patients had any other disease been found which appeared to have a bearing on the diagnosis.

#### *Vestibular habituation*

Habituation, the facility of not responding to or regulating repetitive or unwanted stimuli, appears to be of considerable importance in vestibular neuronitis and following a severe vestibular disturbance, most notably one of endorgan type the caloric responses may remain severely depressed for long after the vestibular system has returned to normal. In such cases habituation may well of course have been the result of other causes, sport, training or occupation and if the diagnosis of vestibular neuronitis is in any

doubt an enquiry into the patient's history should be made to exclude these causes of habituation. The effects of habituation on the caloric responses may be of long duration. In the present series these responses have in some patients still been absent several years after the initial illness. Severe bilateral depression of caloric responses due to habituation to rotation may be differentiated by rotation tests in darkness or with closed eyes from vestibular neuronitis. Habituation to vestibular stimuli in such persons as figure skaters or ballet dancers is well recognised. Mowrer (1934) and McCabe (1960) were unable to demonstrate a nystagmic response to any form of vestibular stimulation in skaters with eyes open, but if the test were applied in darkness or with eyes lightly closed (Collins, 1966a) vigorous nystagmus occurred which disappeared when the eyes were re-opened in the light. Collins (1966b) found that the speed of the skaters' spin was too great to allow the 'visual spotting' (up to 280 r.p.m.) thus after the sudden stop a few beats of nystagmus were seen, rapidly controlled by visual fixation, but dizziness recurred at once if the eyes were closed. Lidvall (1961) and Gramowski (1964) concluded that the vestibular nystagmic response to repeated stimuli, rotational or thermal, decreased with central training and learning acquired when the stimulus is not required by the brain. Using repeated angular acceleration Lidvall (1961) and Collins & Updegraff (1966) showed that the response decline was fairly specific to a practised direction, affected more the maximum intensity than the duration of nystagmus. This ability to control nystagmus and vertigo is easily and quickly acquired and once acquired may be retained for long periods of time (Hood & Pfaltz, 1954; McCabe 1964, 1966). Patients with peripheral vestibular disorders more easily become habituated and lose their nystagmic response in light to thermal and rotatory stimuli (Caston & Gribenski, 1960) but by rotating such subjects in the dark or with the eyes closed and simultaneously recording the nystagmus it can be shown that their vestibular responses are otherwise normal (Uchytíl & Bochemek, 1960; Mertens & Collins, 1967).

Much variation in the responses to identical rotation in the two directions occurs in normal subjects (Ledoux *et al.*, 1963) and several seconds difference in the responses is not necessarily pathological. In many subjects nystagmus can clearly be seen as the eye moves behind lightly closed lids, giving a useful method of differentiating habituation from true bilateral depression of vestibular thermal responses. The ability to fix the eyes may be reduced by mental arithmetic questions or better still Frenzel's spectacles may be worn. The caloric pattern of unilateral vestibular habituation is a directional preponderance rather than the canal paresis (Hood, 1967) which occurs in bilateral lesions.

#### ACKNOWLEDGMENT

The author would like to acknowledge his indebtedness to Dr J. N. Blau for his many helpful suggestions.

## ZUSAMMENFASSUNG

Eine Anzahl der vestibulären Neuritis ähnlichen Syndrome sind beschrieben alle zeichnen sich dadurch aus, dass Vertigo ihr hauptsächlichstes Symptom ist. In einigen Fällen waren vestibuläre Symptome allein vorhanden und manchmal wurde dann serienweise ein epidemisches Auftreten beobachtet, in andern Fällen fanden wir auch weitere neurologische Symptome, wie z. B. eine Affektion des Oculomotorius. Durch Weiterverfolgen von 60 Fällen mit vestibulärer Neuritis konnten wir zeigen, dass die vestibuläre Neuritis ein abgrenzbares Krankheitsbild darstellt, welches sicher diagnostiziert werden kann. Statistische Tabellen welche Alters- und Geschlechtsverteilung, sowie die Anzahl der Anfälle und ihre Dauer und Stärke enthalten, sind angeführt. Eine schwere vestibuläre Schädigung führt häufig zu einer Gewöhnung eines oder beider vestibulärer Systeme und kann dadurch eine kalorische Unerregbarkeit in einem sonst normalen System bewirken. Eine ähnliche Art der Gewöhnung finden wir auch bei Ballettänzern und Eiskunstläufern, sowie auch in gewissen Berufen. Aus diesem Grunde sollte bei stark verringerter oder nicht auslösbarer kalorischer Reaktion durch genaue Befragung der Patienten abgeklärt werden, ob diese Möglichkeiten einer Gewöhnung bestehen.

## REFERENCES

- ACHAM, G. 1967 Habituation to repeated rotatory stimuli (cupulometry) and the effect of anesthesia drugs and alcohol on the results. *Acta Otolaryng. (Stockh.)* 63, 33.
- ACHAM, G. and ST. NIEL, J. 1966 Vestibular neuritis: Nystagmographic study. *J. Laryng.* 76, 397.
- BARRÉ, J. A. and HENRI, L. 1931 Le Liquide céphalo-rachidien dans l'encéphalite épidémique. *Bull. M. d. (Par.)* 3a, 366.
- BENJAMIN, L. S. 1964 Benign paroxysmal vertigo of childhood (a variety of vestibular neuritis). *Brain*, 87, 131.
- BORR, A. 1963: Cochlear symptoms and signs in vestibular neuritis. *J. Laryng.* 73, 128.
- CAYROL, J. and GIMENEZ, A. 1966 L'influence de la lésion sur le seuil des réactions vestibulaires postrotatoires. *Acta Otolaryng. (Stockh.)* 62, 51.
- COLLINS, W. E. 1966 Problems in spatial orientation: vestibular studies of figure skaters. *Trans. Amer. Acad. Ophthalmol. Otolaryng.* 70, 373.
- 1966 b Vestibular responses from figure skaters. *J. Assoc. Med.* 37, 1098.
- COLLINS, W. E. and LINDHART, R. P. 1966 A comparison of nystagmus habituation in the cat and the dog. *Acta Otolaryng. (Stockh.)* 62, 19.
- DANIELSSON-VALBERG, T. 1953 Further clinical studies on epidemic vertigo, "Vértigo de Vertiginosus". *Acta Psychiat. Scand.* 22, 263.
- DEK, M. P. and HALLPIKE, C. S. 1952 The pathology, symptomatology and diagnosis of certain common disorders of the vestibular system. *Proc. Roy. Soc. Med.* 45, 341.
- ELWORTH, C. W. 1928 *The Ear and Throat*. Leipzig, Berlin.
- ELWORTH, K. H. 1964 The trained labyrinth. *N.Y.D. (Berlin)* 12, 103.
- GRANOWSKA, K. H., LINDER, E. and ENGELHARDT, I. 1966 Clinical aspect of sudden bilateral isolated vestibular disorders. *N.Y.D. (Berlin)* 13, 332.
- HALLPIKE, C. S. 1949 Pathology and differential diagnosis of viral vertigo. *Proc. 15th Int. Congress Otolaryng.*, p. 313.
- HARRISON, M. BRONCK, 1936 Notes on the clinical features and pathology of postcongenital vertigo. *Brain*, 59, 471.

doubt an enquiry into the patient's history should be made to exclude these causes of habituation. The effects of habituation on the caloric responses may be of long duration: in the present series these responses have in some patients still been absent several years after the initial illness. Severe bilateral depression of caloric responses due to habituation to rotation may be differentiated by rotation tests in darkness or with closed eyes from vestibular neuronitis. Habituation to vestibular stimuli in such persons as figure skaters or ballet dancers is well recognised. Mowrer (1934) and McCabe (1966) were unable to demonstrate a nystagmic response to any form of vestibular stimulation in skaters with eyes open but if the test were applied in darkness or with eyes lightly closed (Collins, 1966a) vigorous nystagmus occurred which disappeared when the eyes were re-opened in the light. Collins (1966b) found that the speed of the skaters' spin was too great to allow the "visual spotting" (up to 280 r.p.m.) thus after the sudden stop a few beats of nystagmus were seen, rapidly controlled by visual fixation, but dizziness recurred at once if the eyes were closed. Lidvall (1961) and Gramowski (1964) concluded that the vestibular nystagmic response to repeated stimuli rotational or thermal decreased with central training and learning acquired when the stimulus is not required by the brain. Using repeated angular acceleration Lidvall (1961) and Collins & Updegraff (1966) showed that the response decline was fairly specific to a practised direction affected more the maximum intensity than the duration of nystagmus. This ability to control nystagmus and vertigo is easily and quickly acquired and once acquired may be retained for long periods of time (Hood & Pfaltz, 1954; McCabe 1964, 1966). Patients with peripheral vestibular disorders more easily become habituated and lose their nystagmic response in light to thermal and rotatory stimuli (Caston & Gribsenkl, 1966) but by rotating such subjects in the dark or with the eyes closed and simultaneously recording the nystagmus it can be shown that their vestibular responses are otherwise normal (Uchytíl & Bochemek, 1966; Mertens & Collins, 1967).

Much variation in the responses to identical rotation in the two directions occurs in normal subjects (Ledoux *et al.*, 1963) and several seconds difference in the responses is not necessarily pathological. In many subjects nystagmus can clearly be seen as the eye moves behind lightly closed lids, giving a useful method of differentiating habituation from true bilateral depression of vestibular thermal responses. The ability to fix the eyes may be reduced by mental arithmetic questions or better still Frenzel's spectacles may be worn. The caloric pattern of unilateral vestibular habituation is a directional preponderance rather than the canal paresis (Hood, 1967) which occurs in bilateral lesions.

#### ACKNOWLEDGMENT

The author would like to acknowledge his indebtedness to Dr J. N. Blau for his many helpful suggestions.



## ZUSAMMENFASSUNG

Eine Anzahl der vestibulären Neuritis ähnlichen Syndrome sind beschrieben alle zeichnen sich dadurch aus, dass Vertigo ihr hauptsächliches Symptom ist. In einigen Fällen waren vestibuläre Symptome allein vorhanden, und manchmal wurde dann serienweise ein epidemisches Auftreten beobachtet, in andern Fällen fanden wir auch weitere neurologische Symptome, wie z.B. eine Affektion des Oculomotorius. Durch Weiterverfolgen von 60 Fällen mit vestibulärer Neuritis konnten wir zeigen, dass die vestibuläre Neuritis ein abgrenzbares Krankheitsbild darstellt, welches sicher diagnostiziert werden kann. Statistische Tabellen, welche Alters- und Geschlechterverteilung, sowie die Anzahl der Anfälle und ihre Dauer und Stärke enthalten, sind angeführt. Eine schwere vestibuläre Schädigung führt häufig zu einer Gewöhnung eines oder beider vestibulärer Systeme und kann dadurch eine kalorische Unerregbarkeit in einem sonst normalen System bewirken. Ein ähnlicher Art der Gewöhnung finden wir auch bei Ballettstartern und Eiskunstläufern sowie auch in gewissen Berufen. Aus diesem Grunde sollte bei stark verringerter oder nicht auslösbarer kalorischer Reaktion durch genaue Befragung des Patienten abgeklärt werden, ob diese Möglichkeiten einer Gewöhnung bestehen.

## REFERENCES

- Aschan, G. 1947 Habituation to repeated rotatory stimuli (cupulometry) and the effect of nitrazene drugs and alcohol on the results. *Acta Otolaryng. (Stockh.)* 61, 55.
- Aschan, G., and S. Axelson, J. 1958 Vestibular neuritis. Nystagmographical study. *J. Laryng.* 78, 497.
- Barré, J. A., and Riva, L. 1921 Le Liquide céphalo-rachidien dans l'encéphalite épidémique. *Bull. Méd. (Par.)* 25, 348.
- Bauer, L. S. 1944 Deafness paroxysmal crisis of childhood (acute of vestibular neuritis). *Brain*, 67, 341.
- Borg, A. 1945 Cochlear symptoms and signs in vestibular neuritis. *J. Laryng.* 73, 336.
- Caston, J., and Greenberg, A. 1946 L'importance de la vision sur le sens des réactions vestibulaires posturales. *Acta Otolaryng. (Stockh.)* 62, 54.
- Collins, W. E. 1946 Problems in spatial orientation: vestibular studies of figure skaters. *Trans. Am. Acad. Ophthalm. Otolaryng.* 48, 52.
- 1946 b Vestibular responses from figure skaters. *Laryngol. Med.* 37, 1065.
- Collins, W. E., and L. F. Dugan, B. P. 1946 A comparison of nystagmus habituation in the cat and the dog. *Acta Otolaryng. (Stockh.)* 62, 19.
- Dalsgaard-Andersen, T. 1933 Further clinical studies on episodic vertigo. "Labyrinthitis Vertiginosa." *Acta Psychiat. Scand.* 28, 263.
- Dei, M. H., and Hallpike, C. S. 1952: The pathology, symptomatology and diagnosis of certain common disorders of the vestibular system. *Proc. Roy. Soc. Med.* 45, 341.
- Elovaara, C. O. 1929 *Die Encephalitis Lethargica*. Berlin.
- Greenberg, A. H. 1941 The isolated labyrinth. *N.Y.O. (Berlin)* 12, 185.
- Greenberg, A. H., L. S. Bauer, E., and E. W. Dugan, J. 1946 Clinical aspects of sudden unilateral isolated vestibular disorders. *N.Y.O. (Berlin)* 14, 332.
- Hallpike, C. S. 1919 Pathology and differential diagnosis of acute vertigo. *Proc. R. Soc. Med.* 12, 336.
- Harrison, M. Spencer, 1936: Notes on the clinical features and pathology of postcongestional vertigo. *Brain*, 59, 14.

doubt an enquiry into the patient's history should be made to exclude these causes of habituation. The effects of habituation on the caloric responses may be of long duration. In the present series these responses have in some patients still been absent several years after the initial illness. Severe bilateral depression of caloric responses due to habituation to rotation may be differentiated by rotation tests in darkness or with closed eyes from vestibular neuronitis. Habituation to vestibular stimuli in such persons as figure skaters or ballet dancers is well recognised, Mowrer (1934) and McCabe (1960) were unable to demonstrate a nystagmic response to any form of vestibular stimulation in skaters with eyes open, but if the test were applied in darkness or with eyes lightly closed (Collins, 1966a) vigorous nystagmus occurred which disappeared when the eyes were re-opened in the light. Collins (1966b) found that the speed of the skaters' spin was too great to allow the "visual spotting" (up to 289 r.p.m.) thus after the sudden stop a few beats of nystagmus were seen, rapidly controlled by visual fixation, but dizziness recurred at once if the eyes were closed. Lidvall (1961) and Gramowski (1964) concluded that the vestibular nystagmic response to repeated stimuli, rotational or thermal, decreased with central training and learning acquired when the stimulus is not required by the brain. Using repeated angular acceleration Lidvall (1961) and Collins & Updegraff (1966) showed that the response decline was fairly specific to a practised direction, affected more the maximum intensity than the duration of nystagmus. This ability to control nystagmus and vertigo is easily and quickly acquired and once acquired may be retained for long periods of time (Hood & Pfaltz, 1954; McCabe 1964, 1966). Patients with peripheral vestibular disorders more easily become habituated and lose their nystagmic response in light to thermal and rotatory stimuli (Caston & Gribenski, 1960) but by rotating such subjects in the dark or with the eyes closed and simultaneously recording the nystagmus it can be shown that their vestibular responses are otherwise normal (Uchytel & Bocheniek 1966; Vertens & Collins, 1967).

Much variation in the responses to identical rotation in the two directions occurs in normal subjects (Ledoux *et al.*, 1963) and several seconds difference in the responses is not necessarily pathological. In many subjects nystagmus can clearly be seen as the eye moves behind lightly closed lids, giving a useful method of differentiating habituation from true bilateral depression of vestibular thermal responses. The ability to fix the eyes may be reduced by mental arithmetic questions or better still Frenzel's spectacles may be worn. The caloric pattern of unilateral vestibular habituation is a directional preponderance rather than the canal paresis (Hood 1967) which occurs in bilateral lesions.

#### ACKNOWLEDGMENT

The author would like to acknowledge his indebtedness to Dr J. N. Blau for his many helpful suggestions.

## ADAPTATION TO PROLONGED CONSTANT ANGULAR ACCELERATION

J. H. BROWN and J. W. WOLFE

*From the U.S. Army Medical Research Laboratory Fort Knox, Ky U.S.A.*

Two independent groups of normal human subjects were exposed to a number of long-duration (up to 96 sec) relatively high-intensity ( $3 / \text{sec}^2$  to  $24 / \text{sec}^2$ ) constant, angular accelerations. Nystagmic decrements during stimulation were clearly evident. The decrements were initiated at about the same time after stimulus onset (28-32 sec) for all accelerations used. The decrements in the nystagmic responses were compared to related findings for both subjects' and electrophysiological responses.

Response measures of stimulation of the vestibular apparatus may be organized into three general categories: (1) reflexive eye-movements (e.g. vestibular nystagmus and counterrolling); (2) electrophysiological recordings; and (3) subjective measures of angular velocity including both indirect measures such as the oculogyral (Graybiel & Hupp, 1946) oculogravic (Graybiel, 1952) and audiogyral (Arnault, 1950) illusions, and more direct psychophysical measures such as numerical magnitude estimation (Brown, 1966) and key press estimates (Guedry 1963) of velocity.

One of the most meaningful approaches to the understanding of vestibular function is the systematic comparison of these different responses during similar stimulus manipulations (e.g. Collins & Guedry 1962; Crampton, 1963, 1968). This approach provides an emphasis on interactions of the vestibular system with other sensory systems, thus facilitating integration and meaningful application of data, as well as defining the most important future research problems. For example, although the various intersensory illusions resulting from exposure to accelerative stimulation have been extensively studied, the pervasive influence of vestibular stimulation upon other sensory systems responses does not appear to have been generally appreciated. However, when electrophysiological, nystagmic, and subjective responses to accelerative stimulation are jointly evaluated, it appears that the cerebellum is intricately involved in both the organization of vestibular inputs and the integration of vestibular information with other sensory systems (Wolfe 1968). Hoella (1962, p. 113) points out that "The cerebellum with its intense supply of vestibular proprioceptive, tactile, optic, and acoustic impulses certainly is a structure uniquely capable of integrating these afferent signals. There is good electrophysiological evidence that convergence of heteromodal volleys does occur in the cerebellum." Clearly, determination of the basic mechanisms involved must include an integration of data from these three general response categories.

- 1962 Epidemic vertigo-vestibular neuronitis. A clinical study *Brain*, 85, 612.
- 1966: *The vestibular system and its diseases* p. 403 Univ of Pennsylvania Press.
- HART W J C., 1965 Vestibular paralysis. *Ann. Otol* 74 33.
- HEMMERT, G. 1954: Post infectious meningo-encephalitis as etiologic factor in certain cases of vertigo. *Acta Otolaryng* (Stockh.) 43 Suppl. 118 109
- HOOD, J D 1967 Personal communication
- HOOD J D and PFALTZ, C. R. 1964 Observations upon the effects of repeated stimulation upon rotational and caloric nystagmus. *J Physiol* 123 130
- LEDoux, A LAUWER, P and LEWANS, P 1963: La valeur clinique de l'épreuve rotatoire *Acta Otorhinolaryng Belg* 17 403.
- LEISHMAN, A W D 1955 "Epidemic vertigo with oculo-motor complication. *Lancet* 1 723.
- LIDVALL, H F 1961 Vertigo and nystagmus responses to caloric stimuli repeated at short intervals. *Acta Otolaryng* (Stockh.) 53 33.
- LINDSAY J R and HEMENWAY W E 1956 Postural vertigo due to unilateral sudden partial loss of vestibular function. *Ann Otol* 65 892.
- MCCANE, B. F 1960 Vestibular suppression in figure skaters. *Trans Amer Acad Ophthalmol laryng.*, 64 284
- MCCANE, B. F and GULLINGHAM, K., 1964 The mechanism of vestibular suppression. *Trans. Amer Otol Soc* 59 226
- 1964 The mechanism of vestibular suppression *Ann. Otol* 3 818
- MEHMEKE, S 1963 Zur Klinik der Neuronitis Vestibularis. *Z Laryng Rhinol Otol* 42 679
- MERFIELD, D O 1965 Self limited idiopathic vertigo. *Arch Otolaryng* (Chic.) 81 333.
- MERTENS, R A and COLLINS, W E., 1967: Unilateral caloric habituation of nystagmus in the cat. Effect on rotational and bilateral caloric responses. *Acta Otolaryng* (Stockh.) 64 231
- MOWBRAY, O H 1934: Influence of "exhaustion" on duration of post rotational nystagmus *Arch. Otolaryng* (Chic.) 19 46.
- PEDERSEN, E., 1959: Epidemic vertigo-clinical picture pathology and relation to encephalitis. *Brain*, 82 666.
- PFALTZ, C. R 1955: Diagnose und Therapie der vestibulären Neuronitis *Pract Otorhinolaryng* (Basel) 17 454
- PFALTZ, C. R and von ARX, S 1967 Zur Wirkung wiederholter thermischer und rotatorischer Reize auf das normale vestibuläre Endorgan *Acta Otolaryng* (Chic.) 63, 191
- POSTON, R I 1928: Vestibular labyrinthine epidemic encephalitis *Brain*, 50 482.
- STAHL, J 1966 *The vestibular system and its diseases* p 439 Univ of Pennsylvania Press.
- THOMPSON V E. A., and BERNSTEIN E. C., 1963 Neuronitis vestibularis in brucellosis. *Pres. Med Agent* 50 790
- UCHIYAMA, B and BOCHNER, R. Z., 1966 Vestibular habituation *C S Otolaryng* 15 333.
- WALFORD, P A 1952 An unusual picture. *Lancet* 7 415
- DE WEESE, B D 1954: *Dizziness An evaluation and classification* C. C. Thomas, Springfield, Ill
- WILLIAMS, S 1963 Epidemic vertigo in children *Med. J Aust et al* 50 680
- WINTNER, E., 1952 Epidemic vertigo (neuronitis vestibularis) *gesk Laryng* 114 1363.
- WLODKA, J 1967: Neuronitis vestibularis. *Arch Otolaryng* (Chic.) 83 298
- WORTER DROUGH C., 1952: An unusual epidemic. *Lancet* 1 371

M Spencer Harrison M.D  
The National Hospital of Nervous Diseases  
Queen's Square London W1C England

Received May 27 1968

& Crampton, 1964) Constant angular accelerations of precise duration and intensity were programmed from control equipment outside the room which housed the accelerator. The subject was enclosed in a light tight removable capsule. Electronic voice communication through slip rings permitted continuous contact with *S*. An adjustable bite-board maintained *S*'s head in a fixed position over the axis of rotation.

### *Recording*

Electrodes were taped near the outer canthi of the eyes with an indifferent electrode placed in the center of the forehead. EOG signals for summated horizontal eye movements were led through slip rings to a Grass-ink writing recorder in the adjacent control room. Eye movement potentials were amplified with a 30-sec. RC, time constant and displayed with a 25 mm/sec paper speed. For calibration purposes three lights were placed in front of *S* such that the left and right light subtended a 10° visual angle from the center. Prior to each *S*'s first trial and again following his last, the lights were turned on. A 10° voluntary movement of the eyes was recorded to obtain a calibration relating actual angular displacement of the eyes to mm of recorded eye movement. Periods of acceleration also were recorded.

### *Procedure for Experiment 1*

All testing was conducted in total darkness and each *S* was instructed to keep his eyes open and generally directed straight ahead. Fifteen *Ss* were given a counterbalanced series of six experimental trials which consisted of six different angular accelerations: (1) 3°/sec<sup>2</sup> maintained for 88 sec; (2) 5°/sec<sup>2</sup> for 53 sec; (3) 8°/sec<sup>2</sup> for 30 sec; (4) 12°/sec<sup>2</sup> for 20 sec; (5) 17°/sec<sup>2</sup> for 14 sec; and (6) 24°/sec<sup>2</sup> for 10 sec. All accelerations were presented symmetrically around zero velocity from either 20 RPM (8, 12, 17, and 24°/sec<sup>2</sup>) or 22 RPM (3 and 5°/sec<sup>2</sup>) in a CCW direction, through zero velocity to 20 RPM or 22 RPM in a clockwise direction. A preliminary trial of 24°/sec<sup>2</sup> and 10-sec duration provided a check for proper functioning of the recording equipment, and served to acquaint *S* with the experimental procedures. The 15 *Ss* were young men 10-22 years of age with normal labyrinthine function. All *Ss* had been previously exposed to laboratory angular accelerations and were selected for the present study on the basis of their nystagmic records and high motivation. A high level of arousal was maintained by requiring *S* to accomplish mental arithmetic problems during the trials (both successive multiplication and division). Approximately 15 sec before stimulus onset, *S* was assigned a problem which he continued to work until asked for the answer and told to relax by the experimenter. A minimum of 5 min. of constant velocity was programmed between trial 1 decrease the confounding of responses by secondary nystagmus from the previous acceleration.

The biophysical model for the semicircular canals, a torsion pendulum analog (van Egmond *et al.* 1949) has provided a useful model for evaluating the varied responses to angular acceleration (e.g. Meiry 1965 Aschan, 1954). Primary limitations of the model are its inability to handle either the systematic influence of arousal level upon nystagmic responses (e.g. Brown & Crampton, 1964 Collins *et al.* 1961) or the occurrence of extensive adaptation (a response decline during stimulation) during long-duration, constant, angular acceleration. With regard to the question of adaptation, there are apparent discrepancies between subjective and nystagmic responses to angular acceleration. For example subjective responses involving both key press estimates (Guedry 1965) and magnitude estimates (Brown, 1966, 1968 Clark & Stewart 1967) demonstrate extensive adaptation. On the other hand, Collins & Guedry (1962 1967) reported no adaptation in human nystagmic responses to long-duration constant angular accelerations (1.0 and 1.8 /sec<sup>2</sup>) when arousal level was carefully controlled. Other investigators have reported declines in both subjective and nystagmic responses (e.g. Hood 1961 Mowrer 1935) Collins & Guedry (1964) suggest arousal level as a primary explanation for experimental differences with regard to nystagmic adaptation at low intensities, but these authors also discuss the possible involvement of higher threshold, quicker adapting receptor units to account for adaptation at higher intensity levels than they examined. Evidence from electrophysiological recordings from the vestibular nuclei during angular acceleration are ambivalent (Crampton 1965 1966 Jones, 1967). Although the frequency of discharge from some units appeared to decline under prolonged stimulation, most of those sampled did not decline to any significant extent. What decrement is evident might simply be a reflection of overshoot in a non-critically damped receptor system (Cappel, 1966). However since these data were obtained under barbiturate anesthesia, the failure of the units to exhibit any significant adaptation also might be attributed to influences of the anesthetic upon central inhibitory mechanisms (Brazier 1954). In any case, in view of the complete loss of nystagmus under barbiturate anesthesia (Fearing & Mowrer 1934) these data are difficult to interpret relative to adaptation.

The primary purpose of the present studies was to determine the extent of adaptation in vestibulo-ocular reflex responses to long-duration, relatively high intensity constant, angular accelerations, and to compare the magnitude of the decrements with that found for subjective responses to similar stimuli.

## METHOD

### *Apparatus*

#### *Rotatory device*

The rotatory apparatus consisted of an angular acceleration device, driven about the vertical axis by an electrohydraulic motor control system (Brown

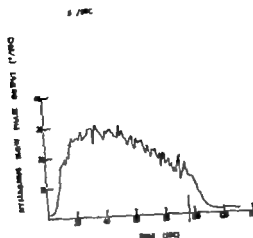


FIG. 1.

FIG. 1—Gyroscope slow phase output (°/sec) for the four angular accelerations. Vertical bars on the baseline define the time of stimulus termination.

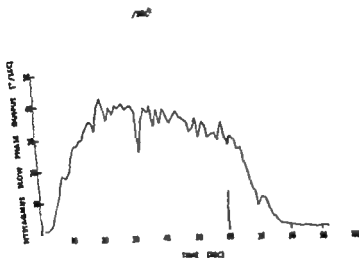


FIG. 2.

### Procedure for Experiment 2

The method and procedures were essentially the same as that in experiment 1 with the exception that all stimulus durations were maintained as far as feasible within the limitations of maximum RPM. All accelerations were presented symmetrically around zero velocity from 40 RPM in a CCW direction to 40 RPM in a CW direction. Each of 10 experienced subjects received four angular accelerations in a partially counterbalanced order: (1)  $1 \text{ sec}^2$  96 sec (2)  $8 \text{ sec}^2$  60 sec (3)  $10 \text{ sec}^2$  48 sec and (4)  $12 \text{ sec}^2$  40 sec. Arousal level was maintained by mental arithmetic tasks.

### *Results and Discussion — Experiment 1*

Nystagmic responses for all trials except the preliminary trial were scored by hand. The vertical magnitude of the slow phase sweep of each primary nystagmic beat was measured in mm for each 1 sec segment of the record, and then converted to degrees of slow phase by means of the calibration taken at the end of each session. Logarithmic transformations of each *S*'s nystagmic output for each second of every trial were made the logs then summed across *S*s, and geometric means computed for each second of each of the six trials.

The averaged, second by second, slow phase output is plotted for the six angular accelerations. Adaptation (a decline in output during the period of stimulation) is clearly evident in responses to the longer duration, lower intensity accelerations ( $3/\text{sec}^2$  and  $5/\text{sec}^2$ ). Despite the considerable second-to-second variation in the nystagmic responses (a typical finding when slow phase is plotted in this manner) lines of best fit drawn by eye for these two accelerations have a negative slope from the points of peak output at approximately 35 sec of stimulation. However this decline must be described as only moderate, as compared to the nearly complete adaptation found in subjective estimates of angular velocity to similar accelerations (Brown 1966 1968).

It is interesting to note that the nystagmic decline from peak output was initiated at approximately the same time (35 sec after the onset of acceleration) for both stimuli in spite of the much longer duration of the  $3/\text{sec}^2$  stimulus. None of the other stimuli were maintained past this apparently critical point for onset of nystagmic adaptation, and no adaptation was evident. It would be of considerable significance to determine if the time of onset of adaptation is in fact common to a wide range of angular accelerations. To the extent that this could be demonstrated, vestibular adaptation would not appear to be a phenomenon of peripheral origin. Adaptation to sensory stimulation has been traditionally viewed as a peripheral not a central phenomenon (Rushton, 1961). But in terms of the mechanical characteristics of the semicircular canals, it is highly unlikely that adaptation to constant angular acceleration reflects changes in the dynamics of the cupula-endolymph system (van Egmond *et al.*, 1949). Löwenstein's work with the ray (1956) indicates that adaptation could have a peripheral locus since different neural units apparently are initiated during cupula deflection and higher threshold units appear to adapt more readily. If in fact this were the mechanism for adaptation response declines during prolonged stimulation should be more quickly initiated and have a steeper slope with higher intensity stimuli. That is, after 30–35 sec, the cupula would be deviated considerably more and the assumed higher threshold faster adapting units more quickly brought into play for an acceleration of  $12/\text{sec}^2$  than for one of  $3/\text{sec}^2$ . Experiment 2 was designed to help answer this question.



TABLE 1 Slopes and Intercepts from nystagmic peak  
Output to stimulus termination.

Output to stimulus termination			
Acceleration (/sec)	Least squares slope	Least squares intercept	Actual peak output
5	25	30.8* at 32 sec	29.6 at 32 sec
8	25	38.9 at 28 sec	40.5 at 28 sec
10	92	46.1 at 30 sec	48.8 at 30 sec
12	81	51.1 at 30 sec	51.6 at 30 sec

er it would appear that the onset of the decrement is initiated at approximately the same time following stimulus onset (28-32 sec) apparently independent of the intensity of the stimulus.

In both experiments a nystagmic decrement was evident during acceleration. Further this decrement was initiated at about the same time for all accelerations used and, the more intense the acceleration, the greater the slope of decrement. Although it is conceivable that the reported decrements are due to decreases in arousal level during stimulation, as Collins & Guedry (1962) suggest, in view of both the assigned mental arithmetic tasks and the relatively large decrements found, arousal level would not appear to be a satisfactory explanation of the phenomenon. The manipulation of arousal level evidently can compensate for "adaptation" of nystagmic responses to low intensity accelerations (Collins & Guedry 1962) yet, a decrement is evident despite high arousal level in response to more intense stimuli if the acceleration are maintained past an apparently critical duration of 30-35 sec.

This close time-locked relationship between the onset of nystagmic adaptation and duration of stimulation, which appears to be essentially independent of stimulus intensity is difficult to explain. However the same general time relations are evident in the onset of adaptation for subjective responses (Brown, 1966, 1968; Clark, 1968) i.e., adaptation occurs only after 20-35 sec of stimulation, regardless of the intensity of the acceleration. Both subjective and nystagmic responses appear to be reflecting a generally similar course for onset of a decline during protracted stimulation.

Wolfe (1968) has shown that infra-slow potentials from the cerebellar vermis of cat reflect peaks which may vary in period from as short as 10 sec to as long as 3 minutes. Significant variables appear to be how much stimulation has been received and arousal level. As shown in Fig. 2, in cats there is a slow conjugate shifting of the eyes in addition to the nystagmic response which seem to bear a relationship to these infra-slow vermal potential (Wolfe 1968). Slow but systematic phase displacements of the eyes are not uncommonly encountered in human EOG recordings, and,

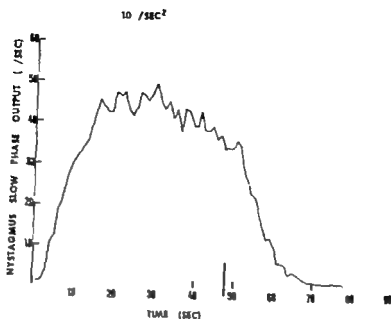


FIG. 3.

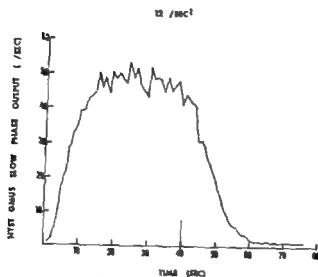


FIG. 4.

### *Results and Discussions for Experiment 2*

Again, geometric means were computed for slow phase output for each second of the four accelerations. These data are shown in Figs. 1-4. Several aspects of these data require comment. A significant response decline during periods of stimulation is clearly evident in nystagmic responses to each of the accelerations. The slope and intercept of each of these four decrements, from actual peak output to stimulus termination, were calculated by the method of least squares. These data are summarized in Table 1. The general trend is for the slopes to become more negative as the intensity of the acceleration increases. These data tend to indicate that once initiated, nystagmic output declines more quickly for more intense accelerations. How

## RESUME

Deux groupes indépendantes des hommes normaux comme les sujets d'essai ont été exposées au nombre des accélérations de la longue durée (à 96 sec) de la latéralité relativement élevée ( $3 \text{ sec}^{-2}$ - $24 \text{ sec}^{-2}$ ) constantes et angulaires. Les diminutions nystagmiques pendant la stimulation ont été observées et clairement claires. Les diminutions ont été commencées approximativement la même temps après du commencement de la stimulation (23-22 sec) pour toutes les accélérations employées. Les diminutions dans les réponses nystagmiques ont été comparées avec les résultats relatifs pour les réponses subjectives et électrophysiologiques.

## ZUSAMMENFASSUNG

Zwei unabhängige Gruppen von normalen Menschen als Versuchspersonen wurden zur Anzahl von langdauernden (bis zu 96 sec) verhältnismäßig höchst intensen ( $3 \text{ sec}^{-2}$  -  $24 \text{ sec}^{-2}$ ) konstanten, winkligen Beschleunigungen ausgesetzt. Nystagmische Verminderungen während der Reizung waren klar offensichtlich. Die Verminderungen wurden ungefähr um dieselbe Zeit nach dem Reisanfang (23-22 sec) begonnen, für alle gebrauchten Beschleunigungen. Die Verminderungen in den nystagmischen Antworten wurden verglichen mit den in Beziehung zueinander stehenden Befunden für subjektive und elektrophysiologische Erwidern gen.

## REFERENCES

1. von M. D. 1940 Post-rotatory localization of sound. *Amer J Psychol* 63, 229.  
 ASCHAN, G. 1954 Responses to rotary stimuli in fighter-pilots. *Act Otolaryng* (Stockh.) 5 pp. 118.  
 BRADLEY, M. A. B. 1951 In *Brain mechanisms and consciousness* (ed. E. D. Adrian et al.) Thomas, Springfield, Ill.  
 GAO, C. J. H. 1964 Magnitude estimation of angular velocity during passive rotation. *J Exp. Psychol.* 9 169.  
 — 1965 Cross-modal estimation of angular velocity. *Percept. Psychophys.* 3 (3A) 112.  
 GAO, C. J. H. and CHAN, C. K. H. 1964 Quantification of the human nystagmic response to angular acceleration. Prediction formulae and monograph. *Act Otolaryng* (Stockh.), 48 333.  
 CARROLL, W. L. 1966 Determination of physical constants of the semicircular canal from measurement of angular velocity by means of constant angular acceleration. In *2nd symposium on the role of the vestibular organs in the perception of motion* pp. 229-236. NAS.  
 CLARK, E. and S. W. J. D. 1967 Magnitude estimates of rotational velocity during and following prolonged increasing constant and to angular acceleration. *Amer Psychol Center* NAS, Moffett Field, Calif.  
 LALLI, A. W. E., CHAN, C. K. H., and FONG, J. D. 1961 Effects of mental activity on vestibular system and the electroencephalogram. *Nature* 190 184.  
 LALLI, A. W. E., and GAO, C. J. H. 1962 Arousal effects and nystagmus during prolonged constant angular acceleration. *Act Otolaryng.* (Stockh.) 51 249.  
 — 1967 Frequency and subjective turning responses to durations of angular acceleration. *FAA JAG* 6-6.

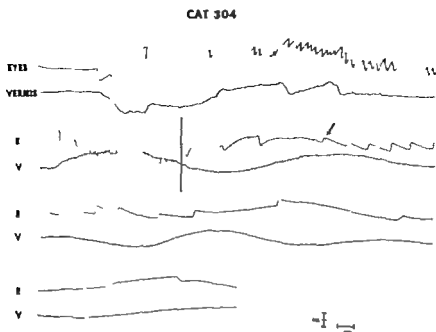


FIG. 5. Record of optokinetic nystagmus indicating infraslow deviations of the eyes. Note that the period of the infra-slow cerebellar potential closely approximates that of the EOG. The arrow denotes the onset of a secondary optokinetic nystagmus. Both the EOG and the cerebellar potential flatten as the optokinetic response drops at EOG = 500  $\mu\text{V}/\text{cm}$ , vermis 200  $\mu\text{V}/\text{cm}$ , 3 sec. T.C.; 25 mm/sec.

furthermore, Clark & Stewart (1967) have reported a similar "waxing and waning" of subjective responses during long-duration angular acceleration. One might speculate that either the vermal potential or the phase displacement of the eyes could provide the necessary neural activity to initiate a response decline. Exploration of this intriguing possibility will require comparison of cerebellar potentials and infra-slow eye movements with the nystagmic and subjective decrements.

In conclusion, even though nystagmic responses do show a clear decrement during constant angular acceleration yet the vestibulo-ocular reflex does not decline as rapidly or as much as do subjective responses to comparable accelerations (Brown, 1966, 1968). In addition to "adapting" more quickly subjective responses also appear to "habituate" and recover from habituation more quickly than do nystagmic responses (e.g. Aschan 1954, Griffith, 1924, Guedry *et al.* 1961). In view of the previous discussion it is tempting to speculate further that the response decline during stimulation operationally a classical example of adaptation, is, in fact, only another reflection of a central habituation process.

#### ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of D. A. Roberts.

## AURAL HARMONICS THE TONE-ON-TONE MASKING VS. THE BEST BEAT METHOD IN NORMAL AND ABNORMAL LISTENERS

T. D. CLACK and F. H. BESS

*From the Kresge Hearing Research Institute, University of Michigan Medical School and the Speech and Hearing Clinic, University of Michigan, Ann Arbor Mich., U.S.A.*

Amplitude distortion is revealed through the generation of harmonics when a pure tone signal of sufficient intensity is presented to the ear. The psychophysical procedure most commonly used to detect the onset of aural harmonics, called the threshold of the aural harmonic, is the method of best beats. This technique, however, is difficult to use clinically. Complex time-consuming judgements are required and the obtained measurement has an overestimation bias due to masking. A tone-on-tone masking procedure is described and suggested as a simpler alternative for obtaining the same information.

Two experiments have been performed to demonstrate the similarity between the harmonic threshold and the masking threshold. In the first, the thresholds of masking at one octave above the fundamental ( $\sim 1000$  Hz) and the second harmonic thresholds are shown to be within a few dB SL of each other in normal ears. In addition, both techniques tend to rank the two ears of the same listener in a similar way. The second study shows that the masking thresholds for a small sample of abnormal listeners are lower than for a comparable group of normals. Also, the mean masking threshold of the abnormal, 28 dB SL, is within the range of harmonic thresholds, 13 to 30 dB SL, reported by previous investigators. Thus, these preliminary results indicate that the diagnostic information obtained by the tone-on-tone masking technique is equivalent to the harmonic thresholds measured by the best-beat method. The masking procedure is both simpler and quicker than the best-beat method and, therefore, more practical for use in the clinic.

Pure tone stimulation at sufficient intensities produces perception of the signal's pitch, loudness, and two less directly observable events—harmonic distortion and masking. The ear generates overtones, called aural harmonics (AHs) when the mechanisms within the cochlea (Wever & Lawrence 1954) are forced to vibrate beyond their capacity for simple proportionate response. At the same time the fundamental signal also causes the ear to lose

This investigation supported by PHS research grant NB 03735 and NB 07242 from the National Institutes of Neurological Diseases and Blindness. Both experiments have been reported before the American Speech and Hearing Association at the annual convention (1966 and 1967).

- CRAMPTON, G. H. 1965. Response of single cells in cat brain stem to angular acceleration in the horizontal plane. In *1st symposium on the role of the vestibular organs in the exploration of space*, pp. 85-98. NASA Report SP 77.
- 1966. Does linear acceleration modify cupular deflection? In *2nd symposium on the role of the vestibular organs in the exploration of space*, pp. 169-184. NASA Report SP 115.
- VAN EDKOND, A. A. J. GROEN, J. J. and JONGHEER, L. H. W. 1949. The mechanics of the semicircular canal. *J. Physiol.* 110: 1.
- FEARING, F. and MOWMAN, O. H. 1934. The effect of general anesthesia upon the experimental reduction of vestibular nystagmus. *J. Gen. Psychol.* 11: 133.
- GRAYBIEL, A. 1952. The oculogravic illusion. *Arch. Ophthalmol. (Chic.)* 48: 605.
- GRAYBIEL, A. and HUFF, D. L. 1946. The oculogyral illusion. *J. Aviat. Med.* 17: 2.
- GRIFFITH, C. H. 1924. A note on the persistence of the practice effect in rotation experiments. *J. Comp. Psychol.* 4: 137.
- GURDY, F. E., 1965. Psychophysiological studies of vestibular function. In *Contributions to sensory physiology* (ed. W. D. Neff) p. 63. Academic Press, New York.
- GURDY, F. E., COLLINS, W. E., and SHEFFER, P. L., 1961. Perceptual and oculomotor reactions to interacting visual and vestibular stimulation. *Percept. Motor Skills* 12: 307.
- HOOD, J. D. 1961. The neurophysiological significance of cupular adaptation and its significance upon Ewald's second law. *Acta Otolaryng. (Stockh.)* Suppl. 159.
- JONES, G. M. 1967. On the dependence of oculomotor response upon the neural response of the vestibular system. In *3rd symposium on the role of the vestibular organs in space exploration*. NASA Report, vol. 3. Pensacola, Florida.
- KORILA, W. P. 1962. Organizational aspects of some subcortical motor areas. *Int. Rev. Neurobiol.* 4: 71.
- LÖWENSTEIN, O. 1956. Peripheral mechanisms of equilibrium. *Brit. Med. Bull.* 12: 114.
- MERRY, J. L., 1965. *The vestibular system and human dynamic space orientation*. Doctoral dissertation. Dept. of Aeronautics and Astronautics.
- MOWMAN, O. H. 1935. The nystagmic response of the pigeon to constant angular acceleration at liminal and supraliminal intensities. *J. Comp. Psychol.* 19: 177.
- RUSHTON, W. A. H. 1961. Peripheral coding in the nervous system. In *Sensory communication* (ed. W. A. Rosenblith) p. 169. Wiley, New York.
- WOLFE, J. W.: Evidence for cerebellar control over habituation of vestibular nystagmus. *Acta Otolaryng. (Stockh.)* Suppl. In press.

US Army Medical Research  
Laboratory Fort Knox Ky 40121  
U.S.A.

Received May 31 1968

## AURAL HARMONICS THE TONE-ON-TONE MASKING VS. THE BEST BEAT METHOD IN NORMAL AND ABNORMAL LISTENERS<sup>1</sup>

T. D. CLACK and F. H. BESS

*From the Kresge Hearing Research Institute University of Michigan Medical School, and the Speech and Hearing Clinic University of Michigan, Ann Arbor Mich., U.S.A.*

Amplitude distortion is revealed through the generation of harmonics when a pure tone signal of sufficient intensity is presented to the ear. The psychophysical procedure most commonly used to detect the onset of aural harmonics, called the threshold of the aural harmonic, is the method of best-beats. This technique, however, is difficult to use clinically. Complex time-consuming judgements are required and the obtained measurements have an overall small bias due to masking. A tone-on-tone masking procedure is described and suggested as a simpler alternative for obtaining the same information.

Two experiments have been performed to demonstrate the similarity between the harmonic threshold and the masking threshold. In the first, the threshold of masking at one octave above the fundamental ( $\sim 1000$  Hz) and the second harmonic thresholds are shown to be within a few dB SL of each other in normal ears. In addition, both techniques tend to rank the two ears of the same listener in a similar way. The second study shows that the masking thresholds for a small sample of abnormal listeners are lower than for a comparable group of normals. Also, the mean masking threshold of the abnormal, 26 dB SL, is within the range of harmonic thresholds, 13 to 30 dB SL, reported by previous investigators. Thus, these preliminary results indicate that the diagnostic information obtained by the tone-on-tone masking technique is equivalent to the harmonic thresholds measured by the best-beat method. The masking procedure is both simpler and quicker than the best-beat method and, therefore, more practical for use in the clinic.

Pure tone stimulation at sufficient intensities produces perception of the signal's pitch, loudness, and two less directly observable events—harmonic distortion and masking. The ear generates overtones, called aural harmonics (AH) when the mechanism within the cochlea (Weyer & Lawrence 1954) are forced to vibrate beyond their capacity for simple proportionate response. At the same time the fundamental signal also causes the ear to lose

<sup>1</sup>The investigation was supported by PHS research grant NB 03723 and NB 07252 from the National Institutes of Neurological Disorders and Blindness. Both experiments have been reported before the American Speech and Hearing Association at the annual meetings (1966 and 1967).

sensitivity for the higher frequencies. This spread of masking is presumably the reason aural harmonics are not heard as separate perceptual entities at moderate signal intensities even though their presence is detectable using special psychophysical procedures.

The most common of these procedures is the method of best beats, based upon a suggestion by Wegel & Lane (1924). With this method, clinical investigators have measured the lowest intensity (in dB sensation level) of the pure tone fundamental ( $f_1$ ) required to make the second aural harmonic ( $f_{AH}$ ) just detectable (for review of logic, see esp. Lawrence, 1958). This threshold of distortion has been related to inner ear pathology (Lawrence & Yantis, 1956) to the estimation of cochlear reserve in otosclerotic ears (Yantis & Magielski 1958) to the intelligibility of speech in patients with sensori neural impairments (Yantis *et al.* 1966) and possibly even to the determination of susceptibility to acoustic trauma (Lawrence & Blanchard, 1954; Capano, 1962). In spite of its potential significance in hearing conservation and the diagnostic evaluation of hearing disorders, otologists and audiologists do not utilize the best beat method as a regular clinical tool. This neglect may be due to certain practical as well as theoretical difficulties. One aspect of the latter is the problem of masking at the octave interval and its complicating effect upon resultant measurements.

The confounding effects of sensitivity upon the aural harmonic measurements was first demonstrated and explained by Egan & Klumpp (1951). They suggested that a rather large, somewhat idiosyncratic, bias may be introduced as  $f_1$  intensity is lowered to obtain the aural harmonic threshold. Some more recent work using a different procedure has indicated that the amplitude of  $f_{AH}$  is below the perceptual threshold when the ears distortions begins, and grows at a rate equal to or less than the masked threshold for intensities below about 70 dB SL (Clack, 1967, 1968 *a* 1968 *b*). Such reasoning suggests that the aural harmonic levels, by the best beat method, might be essentially equivalent to the masked threshold measured in the immediate frequency vicinity of  $f_{AH}$ .

To test this hypothesis, two separate experiments are described here. In the first, the thresholds of masking and the aural harmonic threshold levels were obtained from the same sample of normal ears. The second experiment compares the masking thresholds from a group of sensori neural impaired listeners to the aural harmonic thresholds obtained by previous clinical investigators using the best beat method.

## I COMPARISON OF THE TONE-ON-TONE MASKING AND BEST BEAT THRESHOLDS IN NORMALS

### *Method*

#### *Subjects*

Five college students (three male and two female) ranging in age from 22 to 33 with a mean age of 26 years were paid as listeners in this experi-



ment. Each subject was given a sweep frequency interrupted tone, Békésy type audiogram. These observers had audiometric thresholds no greater than 25 dB hearing level (re I.S.O., 1964 norms) at octave frequencies between 125 and 8000 Hz.

### *Best-beats*

The subjects hand held the earphone comfortably against the ear being tested. The listener hears a loud, lower pitched, fundamental with a softer higher pitched, background tone which waxes and wanes in loudness, i.e., beats, at a rate determined by the frequency difference between the aural harmonic and the exploring tone. The intensity of the exploring frequency ( $f$ ) in this case approximately a 2004 Hz tone, is adjusted to maximize the prominence of the beating, obtained initially with a 70 dB SL 1000 Hz fundamental. When the perceptual range of the beat is maximized, as reported by the observer the criterion of "best" is met and the exploring tone is thought to be at the same intensity as the aural harmonic. Both tones were then attenuated together by the experimenter until the beating background could no longer be heard and adjustments of the relative intensity and/or changes in beating rate, due to varying the frequency of the exploring tone, could no longer produce reliable responses. This SL of the fundamental was recorded as the threshold of the aural harmonic.

A Malco Aural Overload Tester MA-3, was used for making measurements by the best-beats method. This apparatus operates effectively as two separate oscillators, one to generate the 1000-Hz fundamental ( $f$ ) and the other to produce  $f$  within a few cycles of the second aural harmonic ( $f_{2H}$ ). The exploring tone can be adjusted separately for "best-beats" at any appropriate intensity of  $f$  and both tones can be attenuated together in minimal steps of 5 dB. Pre-experimental calibration showed that the acoustic harmonic output of the associated ear phone was at least 60 dB below the 1000 Hz maximum  $f_1$  output (Lawrence 1958).

### *Tone-on tone masking*

This procedure simply involves measuring the monaural masking at one octave above the fundamental masker. In these experiments, a continuous  $1000 \pm 1$  Hz signal was the masker and the listener traced his threshold for an interrupted  $2000 \pm 1$  Hz test-tone. The apparatus (Fig. 1) consisted of a Hewlett Packard 203A used to generate the  $f_1$  while the test-tone was generated by the Békésy audiometer. Both  $f$  and the test-tone were monitored with the electronic counter Hewlett Packard 522B, during the tests to keep frequency drift to a minimum. The matching network consisted of two United Transformer Co. LS-33 impedance matching transformers which prevented any measurable feedback between signals. Earphone output was monitored with the coupler and associated instrumentation. A Brüel and Kjaer (Bk) 4134 microphone, a 2801 power supply and a Hewlett Packard

sensitivity for the higher frequencies. This spread of masking is presumably the reason aural harmonics are not heard as separate perceptual entities at moderate signal intensities even though their presence is detectable using special psychophysical procedures.

The most common of these procedures is the method of best beats, based upon a suggestion by Wegel & Lane (1924). With this method, clinical investigators have measured the lowest intensity (in dB sensation level) of the pure tone fundamental ( $f_1$ ) required to make the second aural harmonic ( $f_{AH}$ ) just detectable (for review of logic, see esp. Lawrence, 1958). This threshold of distortion has been related to inner ear pathology (Lawrence & Yantis, 1956) to the estimation of cochlear reserve in otosclerotic ears (Yantis & Maglefski, 1958) to the intelligibility of speech in patients with sensori neural impairments (Yantis *et al.* 1966) and possibly even to the determination of susceptibility to acoustic trauma (Lawrence & Blanchard, 1964; Capano, 1962). In spite of its potential significance in hearing conservation and the diagnostic evaluation of hearing disorders, otologists and audiologists do not utilize the best beat method as a regular clinical tool. This neglect may be due to certain practical as well as theoretical difficulties. One aspect of the latter is the problem of masking at the octave interval and its complicating effect upon resultant measurements.

The confounding effects of sensitivity upon the aural harmonic measurements was first demonstrated and explained by Egan & Klumpp (1951). They suggested that a rather large, somewhat idiosyncratic, bias may be introduced as  $f_1$  intensity is lowered to obtain the aural harmonic threshold. Some more recent work using a different procedure has indicated that the amplitude of  $f_{AH}$  is below the perceptual threshold when the ears distort, and grows at a rate equal to or less than the masked threshold for intensities below about 70 dB SL (Clack, 1967, 1968 a, 1968 b). Such reasoning suggests that the aural harmonic levels, by the best beat method, might be essentially equivalent to the masked threshold measured in the immediate frequency vicinity of  $f_{AH}$ .

To test this hypothesis, two separate experiments are described here. In the first the thresholds of masking and the aural harmonic threshold levels were obtained from the same sample of normal ears. The second experiment compares the masking thresholds from a group of sensori neural impaired listeners to the aural harmonic thresholds obtained by previous clinical investigators using the best beat method.

## I. COMPARISON OF THE TONE-ON TONE MASKING AND BEST BEAT THRESHOLDS IN NORMALS

### *Method*

#### *Subjects*

Five college students (three male and two female) ranging in age from 22 to 33 with a mean age of 26 years were paid as listeners in this experi-

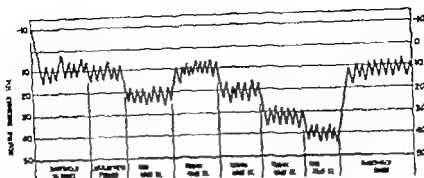


FIG. 2. Typical tracing of the sequence of thresholds obtained from normal ear during one run.

data. After the initial quiet threshold tracing, an interval follows in which the intensity of  $f$  is adjusted by the experimenter usually in 10 dB steps, until a masking level is attained which causes a threshold shift of at least 5 dB. In Fig. 2, for example, the  $f$  at 60 dB SL produced just over a 10 dB threshold shift. The  $f$  was then decreased by 20 dB to find out whether the mere presence of this extraneous stimulus affects the listener's ability to trace his threshold for the exploring tone. The s.d.s. between these tracings and the initial quiet threshold is 1.4 dB. Evidently any threshold shift produced by raising the intensity of the  $f_1$  cannot be attributed to its mere presence. The intensity of  $f_1$  was then increased by 20 dB. The s.d.s. was found to be 1.2 dB between the later and the earlier masked thresholds using this  $f$ -intensity. Obviously the small s.d.s. indicate the reliability of the initial masked threshold levels is quite high. Finally the intensity of  $f_1$  was increased in two steps, usually 5 dB each.

The resulting increases in threshold shift are shown in Fig. 3. These masking shifts appear to grow linearly with only the minor deviations expected in the usual determinations of such thresholds. Using an assumption of linear masking growth, the intensity of  $f_1$  which starts to produce a loss

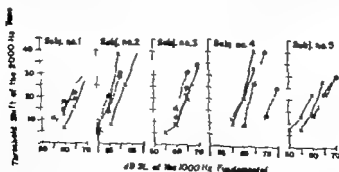


FIG. 3. Threshold shift obtained as the  $f$  intensity is increased in 5 dB steps in separate runs. The masking  $f$  actions from each of five normal listeners are shown with the dashed lines representing the right ear and the solid lines representing the left ear.

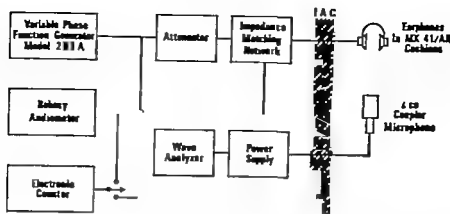


FIG. 1 Electronic equipment used for calibration and measurement of tone-on-tone masking

302A wave analyzer Any acoustic harmonics were at least 60 dB below the level of  $f_1$

The listener was seated in an Industrial Acoustics Corp (I.A.C.) room and the 1000-Hz threshold was determined in the usual clinical manner using the manual attenuator. The listener was then instructed as usual in Békésy type audiometry with the addition of the following "This is the same kind of test you took before (at initial screening) except that the pulsed tone will not change in pitch. After a short time you will hear a different tone, one lower in pitch, that will get louder or softer throughout the test. I shall control this tone and you are to disregard it completely. Continue to listen only to the first tone which may at times sound more like a chirp in the background. Again, you press the button to make this chirping tone disappear as before. Do you have any questions?"

The threshold tracings for the test tone were converted to numerical values by computing the mean of midpoints of the last 10 pen excursions recorded during each period within a test run.

### Procedure

Subjects were assigned randomly to either of two testing sequences in which alternate ears of the listener were tested. In addition, the two psychophysical procedures were used alternately in a balanced manner. All 10 ears were tested twice by both psychophysical techniques.

### Results

A tone-on-tone masking "run" is illustrated with the tracings of Fig 2. The interrupted 2000-Hz test tone threshold is traced at the beginning and at the end of each run. The standard deviation of differences (s.d.) was calculated using the 20 "within run" pairs of thresholds. The quiet threshold s.d. equals 1.4 dB which indicates that no significant fatigue or practice effect was operating, a fact also obvious from inspection of the raw

ing threshold (TOM) 53 dB SL, is slightly lower than the 59 dB harmonic threshold. Apparently masking at the harmonic frequency becomes effective slightly before there is any evidence of amplitude distortion. This finding is consistent with the bias inherent in the best-best measurements (Wegel & Lane, 1924; Egan & Klumpp, 1931; Clark, 1967). The absolute magnitude of the difference, 6 dB, is not, however, directly comparable to the results of previous investigators for two reasons. First, different instructions or testing details, such as attenuating  $f_1$  and the exploring tone ( $f$ ) in steps less than 3 dB, might have resulted in harmonic thresholds differing somewhat from those obtained here. Second, an error is involved in determining the threshold of masking from linear extrapolation. It is well known, for instance, that the growth of masking is S-shaped—not linear (Wegel & Lane, 1924). Thus, it is quite likely that masking actually begins at lower levels than indicated by the TOM values reported here. Although both sources of error affect each of the numerical threshold values to some degree, two facts are clear: (1) masking and harmonic distortion appear at almost the same  $f_1$  levels, and (2) both kinds of threshold measurements tend to rank ears in the same way. This evidence is consistent with the contention that both psychophysical procedures, tone-on-tone masking and the best-best method, produce essentially equivalent results in ears with normal absolute hearing thresholds. What happens when the absolute threshold is affected by sensori-neural disease processes is investigated next.

## II. COMPARISON OF TONE-ON-TONE MASKING THRESHOLDS IN NORMALS AND ABNORMALS

### Method

All apparatus, calibration, and instructions are essentially the same as in the first experiment. The control group consisted of eight normal hearing college students: 14 ears were actually tested. The audiometric threshold of these ears were at most 10 dB hearing level (re I.S.O. 1964 norms) at octave frequencies between 125 and 8000 Hz. The experimental group was composed of eight sensorineural impaired patients (12 ears) obtained from the outpatient audiology clinic at the University of Michigan Hospital. Patient selection was based upon a positive otological history evaluation, and a sensori-neural hearing loss of 20 dB or more at three or more of the audiometric frequencies tested.<sup>1</sup>

### Results

The data needed to calculate the TOM illustrated in Fig. 4, was obtained with a "run" modified from experiment I. Each run consisted, first, of a period of tracing the threshold of the test tone ( $\approx 2000$  Hz) in quiet. There

<sup>1</sup>For clinical review of each patient, see text and appendix.

TABLE 1 *The 2000-Hz masking and overload thresholds obtained in the first and second runs from each of five normal listeners*

Subject number	Ear	Tone-on tone		Best beat		Mean thresholds	
		First	Second	First	Second	Tone-on-tone	Best-beat
1	Right	47	50	55	65	48.5	60.0
	Left	54	57	60	65	55.5	62.5
2	Right	48	47	60	60	47.5	60.0
	Left	34	47	60	60	50.5	60.0
3	Right	33	51	55	55	52.0	55.0
	Left	32	56	55	60	54.0	55.5
4	Right	65	62	60	65	63.5	62.5
	Left	56	56	55	55	56.0	55.0
5	Right	54	52	60	60	53.0	60.0
	Left	51	46	55	55	48.5	55.0
Mean thresholds		53.4	52.4	57.5	60.0	52.9	58.8

of sensitivity in the frequency vicinity of  $f_{AH}$  can be quite easily derived from these data. The two extreme shifts, the highest and lowest points for each run in Fig 3 are connected by a straight line. The line is extrapolated to intercept the abscissa at some  $f_1$  intensity. (This procedure is an expedient made necessary because very slight shifts in the actual threshold tracings cannot be picked out by watching the pen excursions. This simple extrapolation technique removes ambiguity in designating the  $f_1$  intensity which just begins to cause some masking shift of the test tone.) The intercept value is defined throughout the rest of this report as the "threshold of octave masking" (TOM).

Table 1 permits comparison of all the measurements obtained in each ear of each subject by both psychophysical methods. By comparing the two kinds of thresholds between the ears of individuals, it is evident that a lower TOM tends to coincide with a lower best beat threshold, i.e., both measures rank relative thresholds similarly. The largest test-retest difference between aural harmonic thresholds by best beats is 10 dB while TOM has a range of 7 dB. In 8 of 10 ears ( $p < .09$ ) the TOMs are below the best beat thresholds. The mean TOM is about 53 dB for the tone-on-tone technique or about 6 dB below the mean obtained by best beats.

### Discussion

The mean threshold of the aural harmonic, 59 dB SL, is within the range 52 to 65 dB reported by previous investigators (Lawrence & Yantis, 1956; Lawrie & Blanchard, 1954). This sample of normal ears, therefore, seems fairly typical of the population. The mean tone-on-tone mask

ing threshold (TOM) 33 dB SL, is slightly lower than the 39 dB harmonic threshold. Apparently masking at the harmonic frequency becomes effective slightly before there is any evidence of amplitude distortion. This finding is consistent with the bias inherent in the best-beat measurements (Wegel & Lane, 1924; Egan & Klumpp 1951; Clock, 1967). The absolute magnitude of the difference, 6 dB, is not, however directly comparable to the results of previous investigators for two reasons. First, different instructions or testing details, such as attenuating  $f_1$  and the exploring tone ( $f$ ) in steps less than 11 dB, might have resulted in harmonic thresholds differing somewhat from those obtained here. Second, an error is involved in determining the threshold of masking from linear extrapolation. It is well known, for instance that the growth of masking is S-shaped—not linear (Wegel & Lane, 1924). Thus, it is quite likely that masking actually begins at lower levels than indicated by the TOM values reported here. Although both sources of error affect each of the numerical threshold values to some degree, two facts are clear: (1) masking and harmonic distortion appear at almost the same  $f_1$  levels, and (2) both kinds of threshold measurements tend to rank ears in the same way. This evidence is consistent with the contention that both psychophysical procedures, tone-on-tone masking and the best beat method, produce essentially equivalent results in ears with normal absolute hearing thresholds. What happens when the absolute threshold is affected by sensori-neural disease processes is investigated next.

## II. COMPARISON OF TONE-ON-TONE MASKING THRESHOLDS IN NORMALS AND ABNORMALS

### Method

All apparatus, calibration, and instructions are essentially the same as in the first experiment. The control group consisted of eight normal hearing college students. 14 ears were actually tested. The audiometric threshold of these ears were at most 10 dB hearing level (re I.S.O. 1964 norms) at octave frequencies between 125 and 8000 Hz. The experimental group was composed of eight sensorineural impaired patients (12 ears) obtained from the outpatient audiology clinic at the University of Michigan Hospital. Patient selection was based upon a positive otological history evaluation, and a sensori-neural hearing loss of 20 dB or more at three or more of the audiometric frequencies tested.

### Results

The data needed to calculate the TOM illustrated in Fig. 4, was obtained with a "run" modified from experiment I. Each run consisted, first, of a period of tracing the threshold of the test tone ( $\approx 2000$  Hz) in quiet. There

For clinical review of each patient, see text and appendix.

TABLE 1 *The 2000-Hz masking and overload thresholds obtained in the first and second runs from each of five normal listeners*

Subject number	Ear	Tone-on tone		Best-beat		Mean thresholds	
		First	Second	First	Second	Tone-on-tone	Best-beat
1	Right	47	50	55	65	48.5	60.0
	Left	54	57	60	65	55.5	62.5
2	Right	48	47	60	60	47.5	60.0
	Left	54	47	60	60	50.5	60.0
3	Right	53	51	55	55	52.0	55.0
	Left	52	50	55	60	54.0	55.5
4	Right	65	62	60	65	63.5	62.5
	Left	56	56	55	55	56.0	55.0
5	Right	54	52	60	60	53.0	60.0
	Left	51	46	55	55	48.5	55.0
Mean thresholds		53.4	52.4	57.5	60.0	52.9	56.8

of sensitivity in the frequency vicinity of  $f_{MH}$  can be quite easily derived from these data. The two extreme shifts, the highest and lowest points for each run in Fig. 3, are connected by a straight line. The line is extrapolated to intercept the abscissa at some  $f_1$  intensity. (This procedure is an expedient made necessary because very slight shifts in the actual threshold tracings cannot be picked out by watching the pen excursions. This simple extrapolation technique removes ambiguity in designating the  $f_1$  intensity which just begins to cause some masking shift of the test tone.) The intercept value is defined throughout the rest of this report as the "threshold of octave masking" (TOM).

Table 1 permits comparison of all the measurements obtained in each ear of each subject by both psychophysical methods. By comparing the two kinds of thresholds between the ears of individuals, it is evident that a lower TOM tends to coincide with a lower best beat threshold, i.e., both measures rank relative thresholds similarly. The largest test-retest difference between aural harmonic thresholds by best beats is 10 dB while TOM has a range of 7 dB. In 8 of 10 ears ( $p < 0.05$ ) the TOMs are below the best beat thresholds. The mean TOM is about 53 dB for the tone-on-tone technique or about 6 dB below the mean obtained by best beats.

### Discussion

The mean threshold of the aural harmonic, 59 dB SL, is within the range 52 to 65 dB reported by previous investigators (Lawrence & Yantis, 1956; Lawrence & Blanchard, 1954). This sample of normal ears, therefore, seems fairly typical of the population. The mean tone-on-tone mask



TABLE 2. TOMs obtained in two successive runs on each ear of eight normal listeners.

Normal group			
Subject	Ear	First run, dB	Second run, dB
1	R. E.	47	47
G. S.	L. E.	48	47
2	R. E.	45	45
C. C.	L. E.	43	45
3	R. E.	47	43
B. B.	L. E.	43	46
4	R. E.	44	44
R. R.	L. E.	48	45
5	R. E.	48	48
E. S.	L. E.	—	—
6	R. E.	51	51
C. H.	L. E.	54	53
7	R. E.	45	45
J. S.	L. E.	55	57
8	R. E.	46	46
T. M.	L. E.	—	—
Mean		48	48

masking tends to grow either faster or slower in the impaired ear than in the normal. Nonetheless, the mean slope ( $\bar{M}$ ) for the entire group of abnormal, 1.7 dB, is quite similar to that of normals,  $\bar{M} = 1.8$  dB.

The linearity of the masking function, illustrated for three abnormals in Fig. 5, is evident in all the abnormals regardless of the growth rate. Thus, the TOM can be approximated for the abnormals by linear extrapolation. Again, extrapolation is based upon the two extreme masking values as illustrated in Fig. 5, and the TOMs are defined as the intensity of the 1000-Hz ( $f$ ) at which the lines intercept the abscissae. The TOMs obtained in the first and second runs of the normals are presented in Table 2.

The reliability of the TOMs among the normals is obvious from a simple comparison of the first and second runs. The largest difference is only 3.0 dB. The mean thresholds for all 14 ears is 48 dB. This mean is 5 dB lower than the 53 dB derived from the normals measured in the first experiment. Such differences between the means probably reflect simply the individuals sampled since the basic procedures are fairly similar. It is interesting to note, however, that this sample produced masking thresholds about 11 dB (59-48) below the aural harmonic thresholds obtained in experiment I.

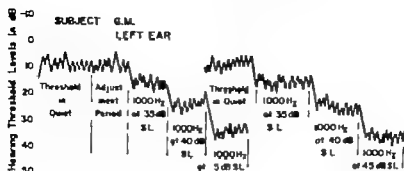


FIG. 4. Typical tracing of the sequence of thresholds obtained from an abnormal ear in each of two successive runs.

follows an interval during which the experimenter increases the 1000 Hz  $f_1$  to produce a threshold shift of at least 5 dB. The  $f_1$  intensity was then increased in two steps, usually 5 dB each. Finally the entire run was repeated with the identical sequence of  $f_1$  intensities. The s.d. for the masked thresholds at each successively higher  $f_1$  intensity level was 2.5 (in quiet) 2.9 2.9 and 2.7 dB for the normals and 1.8 (in quiet) 3.5 2.1 and 2.8 dB for the abnormals, respectively. The test-retest reliability of the masked and quiet threshold for each group is quite good. Also, the reliability for the abnormals approximates that of the normals.

The masked threshold shifts for three selected ears are presented in Fig. 5. These specific threshold shift data were chosen because they span the range of masking slopes ( $M$ 's) which, when averaged over both runs for all 12 abnormal ears, were 0.7 0.8 0.8 0.8, 1.2, 1.2, 1.6 1.9 2.5 2.7 2.8, and 3.0 dB. These slopes can be compared with those from the 14 normal ears which produced  $M$  values of 1.0 1.2, 1.4 1.6 1.8 1.8 1.8 1.8 1.8 1.8, 2.0 2.0 2.6 and 2.6 dB. Notice that better than half (7/12) of the abnormal ears have  $M$  values outside the range (1.0–2.6) of slopes obtained in this sample from normal ears. These distributions of  $M$  values suggests that

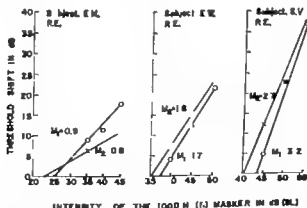


FIG. 5. Threshold shifts obtained as the  $f_1$  intensity is increased in 5 dB steps.  $M$  and  $M'$  are the slopes of two successive runs in the right ear (R.E.) of each of three abnormal ears.

CASE PRESENTATION SUBJECT—R.D.K.  
OTOLOGIC DIAGNOSIS MENIÈRE'S SYNDROME, RIGHT EAR

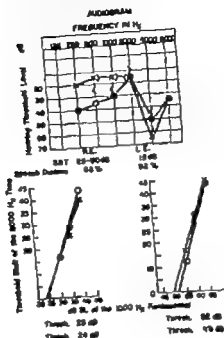


FIG. 6. Preliminary diometric data on subject R. K. together with the tone-on-tone masking data and analysis.

when the right ear was suffering the exacerbation characteristic of Menière's syndrome, the alternate binaural loudness balance (ABLB) showed the presence of recruitment, and short increment sensitivity index (SISI) scores greater than 50% were obtained at all frequencies tested. The important point here is, however, that at the initial visit such evidence of cochlear involvement was not obtained. Although the low frequency thresholds, together with the speech reception thresholds (SRTs) suggest greater involvement in the right ear as compared to the left ear the speech discrimination tests fail to confirm this implication at super threshold levels. The tone-on-tone masking thresholds, however, are approximately 25 dB SL in the right and 50 dB SL in the left. Thus, the TOM procedure clearly differentiates between the functioning of the two ears, even when the more usual speech discrimination testing does not.

There is a distinct practical advantage in substituting the TOM procedure for the best beat method. Often the initial best-beat determinations have taken us as long as 30 to 45 minutes, depending upon the experience, motivation, and intelligence of the listener as well as the experience of the tester. The listener's task is psychophysically quite complex in attempting to maximize the perceptual loudness range of a soft beating tone. In the tone-on-tone masking situation, on the other hand, listeners only determine whether

TABLE 3 *TOMs obtained in two successive runs on eight abnormal listeners*

Abnormal group			
Subject	Ear	First run, dB	Second run dB
1	R. E.	25	24
R. K.	L. E.	52	49
2	R. E.	18	9
R. W.	L. E.	22	23
3	R. E.	57	58
G. M.	L. E.	30	32
4	R. E.	25	20
E. M.	L. E.	No test	No test
5	R. E.	No test	No test
L. R.	L. E.	2	0
6	R. E.	38	36
E. W.	L. E.	No test	No test
7	R. E.	36	35
L. B.	L. E.	31	29
8	R. E.	43	41
S. V.	L. E.	No test	No test
Mean		27	25

Table 3 presents the masking thresholds derived from the first and second runs of all the abnormal ears. There appears to be reasonable reliability among the abnormals. With the exception of two ears, the thresholds differ by 3.0 dB or less on the test-retest basis.

### Discussion

The mean aural harmonic thresholds, reported for groups of abnormals where  $f_1 = 1000$  Hz, are 13 (Yantis & Magielski, 1958) and 30 dB SL (Yantis *et al.*, 1966). The mean TOM, 26 dB SL, obtained with the group of abnormals sampled here falls within this range. Also the mean TOM of the abnormals, 26 dB, is clearly lower than the 48 and 53 dB SLs obtained from comparable measurements in normals. This lowering of the TOMs suggests their interchangeability with aural harmonic thresholds.

The diagnostic relevance of this substitution is evident in a particularly interesting case: R. K., a 35-year-old male jet aircraft mechanic. The routine audiometric thresholds, seen at the top of Fig. 6, indicate a bilateral sensorineural hearing loss caused by noise exposure. The symptoms that brought this patient to the doctor are clear cut in this instance—episodic vertigo, vomiting, with tinnitus and pressure in the right ear. In a subsequent visit

CASE PRESENTATION: SUBJECT—R.D.K.  
OTOLOGIC DIAGNOSIS: MENIÈRE'S SYNDROME RIGHT EAR

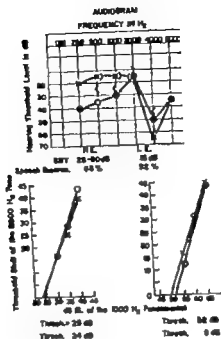


FIG. 6. Preliminary audiometric data on subject R. K. together with the tone-on-tone masking data and analysis.

when the right ear was suffering the exacerbation characteristic of Menière's syndrome, the alternate binaural loudness balance (ABLB) showed the presence of recruitment, and short increment sensitivity index (SISI) scores greater than 50% were obtained at all frequencies tested. The important point here is, however, that at the initial visit such evidence of cochlear involvement was not obtained. Although the low frequency thresholds, together with the speech reception thresholds (SRTs) suggest greater involvement in the right ear as compared to the left ear the speech discrimination tests fail to confirm this implication at super threshold levels. The tone-on-tone masking thresholds, however, are approximately 25 dB SL in the right and 50 dB SL in the left. Thus, the TOM procedure clearly differentiates between the functioning of the two ears, even when the more usual speech discrimination testing does not.

There is a distinct practical advantage in substituting the TOM procedure for the best-beat method. Often the initial best beat determinations have taken us as long as 30 to 45 minutes, depending upon the experience, motivation, and intelligence of the listener as well as the experience of the tester. The listener's task is psychophysically quite complex in attempting to maximize the perceptual loudness range of a soft beating tone. In the tone-on-tone masking situation, on the other hand, listeners only determine whether

or not a tone is present. Although extrapolation of results takes a few minutes, the single sets of threshold shifts needed usually requires less than 5 minutes of patient time. Consequently more patients can be tested more quickly than is possible with the best beat method.

### CONCLUSIONS

In the first experiment the threshold of octave masking (TOM) was shown to be equivalent to the aural harmonic threshold level in normal ears. The second experiment reveals that even when the ear is impaired by sensori neural disease processes, the TOM is affected in the same way as the aural harmonics threshold. It appears that the results of both experiments provide support for the contention that the best beat method and the tone-on tone masking procedure measure essentially the same phenomenon. Furthermore, in at least one case, it was demonstrated that the TOM is more sensitive to super threshold disfunctioning than the usual speech discrimination test. The tone-on tone masking procedure, therefore, appears to have all the essential diagnostic properties which have made measurement of the aural harmonic threshold desirable. Further studies employing larger samples with a wider variety of hearing disorders, however will be needed before a confident judgement can be made about the clinical value of the tone-on tone masking procedure.

### ZUSAMMENFASSUNG

Die Amplitudverzerrung offenbart sich durch die Erzeugung von Obertönen, wenn ein reines Tonsignal von genügender Stärke dem Ohr zugeleitet wird. Der psychophysikalische Vorgang, der meist angewendet wird, um den Beginn von hörbaren Obertönen, genannt die Obertonschwelle, festzustellen, ist die Methode „Best-beats“. Die Methode der Best beats ist jedoch in der klinischen Audiometrie schwierig zu benutzen. Komplex, zeitverzehrende Urteile sind erforderlich, und die erhaltenen Messungen neigen zur Überschätzung aufgrund der Verdeckung. Als ein einfacherer Alternativvorgang zur Erlangung der gleichen Ergebnisse wird ein Ton auf Ton Verdeckungsverfahren beschrieben und empfohlen.

Zwei Versuche wurden unternommen um die Ähnlichkeit zwischen der Obertonschwelle und der Verdeckungsschwelle nachzuweisen. Im ersten wurde gezeigt, dass bei normalem Ohr die Verdeckungsschwelle für einen Ton um eine Oktave höher als die Grundfrequenz ( $\sim 1000$  Hz) und die Schwellen der zweiten Obertöne nur wenige dB SL voneinander getrennt sind. Ausserdem neigen beide Techniken zu einer ähnlichen Reihengliederung der beiden Ohren eines Hörers. Die zweite Untersuchung zeigt, dass die Verdeckungsschwellen einer kleinen Zahl abnormaler Hörer niedriger als die einer vergleichbaren Gruppe normaler Hörer ist. Ausserdem ist die Durchschnittsverdeckungsschwelle der Abnormalen 20 dB SL innerhalb des Obertonschwellenbereiches 13 bis 30 dB SL, wie frühere Forscher berichtet haben. Demnach weisen diese Vorergebnisse daraufhin, dass der kenn-

zeichnende Anschluss, erhalten durch die Ton auf Ton Verdeckungs-technik der Obertonschwächen, gemessen mit der Best-best Methode, gleichwertig ist. Der Verdeckungs-Ver-  
 gang ist einfacher und schneller als die Best-best Methode und deswegen praktischer für klinische Anwendung

## APPENDIX

The following is a clinical description, not previously given in the text, of each abnormal listener sampled in experiment II. Numbers refer to Table 2.

Subject no. 2 (R. W.) is an 18-year-old male, one of three siblings, who have Alport's syndrome. His sensori-neural impairment is mild-to-moderate with fairly good speech discrimination bilaterally. R. W. has been followed for 13 years at this hospital and has no history of vertigo, tinnitus, or other ear disease.

Subject no. 3 (G. M.) is a 41-year-old male who presented with a full sensation and decreased hearing in the left ear. Headache, vertigo, or drainage was not reported and the physical examination was unremarkable.

Audiometric examination revealed a mild bilateral sensori-neural impairment with sharp loss in the higher frequencies (4000-8000 Hz) with the left ear slightly worse than the right. Speech discrimination was good in the right but poor in the left ear. Further assessment of the left ear produced negative SISI scores at 1000, 2000 and 4000 Hz, a clear Type II Békésy audiometric result, and no evidence of tone decay. Unilateral Menière's syndrome was the diagnosis.

Subject no. 4 (E. M.) is a male, 74 years old, with decreased hearing noticed over the previous 10 years. Physical examination was unremarkable.

The audiometric assessment showed a moderate bilateral sensori-neural hearing loss with a threshold decrement for frequencies above 1000 Hz. Speech discrimination was poor. The diagnosis was presbycusis.

Subject no. 5 (L. R.) had a rather sudden onset of hearing loss in the left ear. This 52-year-old male lost all hearing on the left at one time but it gradually returned. He complained of intermittent dizziness as well as the fluctuating hearing. Seven years prior to this examination he had experienced a similar problem in the right ear. A caloric test revealed vestibular function in both ears.

Audiometric evaluation indicated a severe bilateral sensori-neural impairment which was slightly greater at 4000 and 8000 Hz with the right ear somewhat worse than the left. Speech discrimination scores were 68% on the left and only 8% on the right. SISI scores at 1000, 2000 and 4000 Hz were positive in the left ear only. A Type II Békésy audiometric configuration was obtained in the left while the right was a Type I. There was no evidence of tone decay. A diagnosis of Menière's syndrome was made for the left ear.

Subject no. 6 (E. W.) is a 63-year-old male who complained of decreased hearing over a 3-year period. Physical examination revealed nothing unusual.

Audiometry showed mild bilateral sensori-neural hearing loss with a very sharp drop in the higher frequencies beginning at 3000 Hz. Speech discrimination was poor bilaterally. Presbycusis was diagnosed.

Subject no. 7 (L. B.) is a 77-year-old female with progressive hearing loss in the left ear spanning the previous 10 years. There was no history of vertigo but she did complain of dizziness. Physical examination was unremarkable.

Audiometric evaluation revealed a mild bilateral sensori-neural loss centered by

a sharp decrement above 3000 Hz. Her discrimination scores were good bilaterally. Presbycusis was diagnosed.

Subject no 8 (S V) is a 64 year-old male complaining of daily episodes of unsteadiness for a period of 7 months. He described his problem as "the room spinning when his eyes were open. Unsteadiness lasted from 1 to several minutes and was precipitated by turning his head to the right. He reported buzzing heard for 5 years and a pressure sensation.

A moderate bilateral mixed hearing loss was obtained with a depression at 4000 and 8000 Hz. Speech discrimination was fairly good in both ears. The diagnosis was vascular occlusion of the vestibular end artery or viral labyrinthitis.

## REFERENCES

- CAPLAN R. 1962 *Aural distortion and fatigue following exposure to sound*. Unpublished dissertation University of Michigan.
- CLACK, T. DEAN. 1967: Aural harmonic masking of a 2000-Hz tone by sufficient 1000-Hz fundamental. *J Acoust Soc Am* 41, 751.
- 1968a Aural harmonics: Preliminary time-intensity relationships using the tone-on-tone masking technique. *J Acoust Soc Am* 43, 233.
- 1968b Aural harmonics: Tone-on-tone masking at low frequencies of fundamental. Report to 7th Meeting of the Acoust Soc. Am. Ottawa, Canada.
- EGAN, J. and KLUMPF, R. 1951: The error due to masking in measurement of vocal harmonics by the method of best beats. *J Acoust Soc. Am* 23, 275.
- LAWRENCE, M., 1958: Audiometric manifestations of inner ear physiology. The aural overload test. *Trans Amer Acad Ophthalmol Otolaryng* 62, 104.
- LAWRENCE, M. and BLANCHARD, C., 1954: Prediction of susceptibility to noise (trauma) by determination of the threshold of distortion. *Univ Mich Med Bull* 40, 51.
- LAWRENCE, M. and YANTIS, P. 1956: Threshold of overload in normal and pathological ears. *Arch Otolaryng (Chic)* 66, 67.
- OPHEIDT, O. and FLOTTORP, G. 1953: The aural harmonics in normal and pathological hearing. *Acta Otolaryng (Stockh)* 45, 313.
- WHEELER, R. and LANE, C., 1924: The auditory masking of pure tones by a threshold and its probable relation to the dynamics of the inner ear. *Physiol Rev* 23, 266.
- WEVER, E. G. and LAWRENCE, M. 1954: *Physiological acoustics* (Princeton University Press, Princeton, N.J.).
- YANTIS, P. and MACLELLAN, J. 1958: Aural harmonic and bone conduction threshold in the evaluation of cochlear reserve in clinical otosclerosis. *Laryngoscope* 68, 159.
- YANTIS, P., MILLS, J. and SHAPIRO, I. 1966: Speech discrimination in severe sensorineural hearing loss. Two experiments on the role of listening. *J Speech Hear Res.* 9, 18.

Kenneth Haring Research Institute  
University of Michigan Medical School  
Ann Arbor, Michigan U.S.A.

Received July 18, 1968



## THE MIDDLE FOSSA APPROACH FOR REMOVAL OF SMALL ACOUSTIC TUMORS

F. HOUSE and W. E. HITSCHBERGER

*From the Otologic Medical Group, Los Angeles Foundation of Otolaryngology, Los Angeles, Calif., U.S.A.*

We have described a technique for the removal of small acoustic tumors coalescent to, or slightly protruding into the posterior fossa. The case reports of 5 patients have been presented. Total removal of an acoustic tumor was accomplished in all 5 of these patients. The facial nerve was preserved in 3 of the patients and hearing was saved in 4 patients. The study emphasizes the significance of the complaints of vertigo, unsteadiness, tinnitus and mild hearing loss. Patients with these symptoms, either singly or in combination, should receive thorough otologic and radiologic evaluation if they are to receive the maximum benefit from modern neuro-otologic surgery.

The great majority of acoustic tumors arise from the vestibular division of the eighth cranial nerve. Only rarely does the tumor originate from the cochlear division (Crow & Hardy 1938; Skinner 1979). Cochlear deficit results from the pressure by the tumor in the limited confines of the internal auditory canal. We have felt that if the diagnosis of an acoustic tumor could be established while the tumor was small, before severe cochlear nerve damage, tumor removal could be accomplished with preservation or improvement of hearing. The present paper reviews our experience in attempting to accomplish this goal, using a middle fossa approach to the internal auditory canal.

The middle fossa approach to the internal auditory canal was developed in 1938 (House 1961, 1962, 1963) to decompress the internal auditory canal in cases of far advanced otosclerosis. This approach was satisfactory but the decompression had no noticeable effect on the course of the disease.

As experience with this approach increased, it became apparent that the operation could be adapted to the removal of tumors situated in the cerebellopontine angle to reduce the disabling morbidity associated with the conventional suboccipital operation.

Between 1961 and 1963, 22 acoustic tumors were approached through a middle fossa exposure. These patients have previously been reported (House 1964). In these cases, a wide removal of temporal bone to the level of the jugular bulb was carried out through the middle fossa approach.

Sponsored by the Los Angeles Foundation of Otolaryngology

During the same period several operations were performed through the middle fossa approach for the relief of dizziness. In these cases a wide resection of temporal bone was not necessary since the objective was to section the superior vestibular nerve and to preserve the facial and cochlear nerves. In one of these patients a small resectable tumor was discovered in the internal auditory canal. As a result of this, a basic revision of our concepts concerning surgical exposure of these tumors occurred. We felt that the middle fossa approach allowed direct access to the internal auditory canal without necessarily increasing a neurologic deficit. Preservation of facial nerve function and hearing was possible in patients with acoustic tumors if an early diagnosis could be made.

At that time the perfection of surgical technique for the removal of these tumors had advanced beyond our diagnostic capability. Fortunately this dilemma was soon resolved by the development of accurate radiologic techniques for the demonstration of these tumors using positive contrast material (Scanlan, 1964). Recently the introduction of the polytome x ray technique in association with a small aliquot of contrast material has further augmented our diagnostic capability (Hitselberger & House 1967). The detection of tumors in the internal auditory canal is now possible even when they are a few millimeters in diameter. We feel that these tumors represent the ultimate therapeutic challenge to the neuro-otologist using the middle fossa exposure to the internal auditory canal.

## TECHNIQUE OF MIDDLE FOSSA EXPOSURE

### *Preparation of the patient*

The middle fossa approach has been used for removal of small acoustic tumors, facial nerve tumors, and for section of the vestibular nerve in selected cases of advanced Menière's disease. The technique to be described has been used with little modification since 1963.

The operation is performed under light general anesthesia. A supine position is used with the patient's head turned 90° to the side, the involved ear being uppermost. The surgeon is seated at the head of the operating table with the operating microscope on his right and the nurse on his left. A Mayo stand extends over the patient's chest. The anesthetist is positioned at the foot of the operating table. Rubber extension tubing rests on the patient's abdomen and connects the anesthetic machine to the endotracheal tube.

### *Incision and exposure*

The incision extends from a point one finger breadth in front of the tragus at the level of the zygoma, supero-posteriorly to above the helix of the ear. To avoid the fibers of the facial nerve going to the frontalis muscle the incision is kept above the level of the zygomatic arch. A curved incision is preferred to a straight coronal incision to prevent fore shortening of the



FIG. 1 Surgeon uses rectangular shaped bone flap that sides are parallel to facilitate placement of Urban-House retractor

operative exposure. *Blunt finger dissection* is used to develop the plane between the scalp and temporalis muscle, palpating the root of the zygoma and the zygomatic arch. The temporalis muscle is incised along the direction of its fibers from the zygomatic root to its attachment along the supra temporal line. Additional exposure is obtained by incising the temporalis muscle at right angles to the direction of its fibers along the zygomatic root giving an inverted T shaped appearance to the muscle incision. The superficial temporal artery is usually opened and bleeding is controlled by coagulation or suture. Muscle and periosteum are elevated from the underlying calvarium, and self retaining retractors are positioned beneath the muscle.

A rectangular shaped temporal bone flap is turned using the dental drill with cutting burr. A bone flap is preferred over a craniectomy to avoid a cosmetic defect postoperatively. It is important that the opening be rectangular and that the sides are parallel. This facilitates the placement of the Urban House retractor which will be used to elevate the dura overlying the temporal lobe. The base of the bone flap should lie slightly above the zygomatic root, close to the floor of the middle cranial fossa. Its dimensions should be one-third in front of the external auditory canal and two-thirds behind this point. The superior border is at the level of the squamous suture of the temporal bone (FIG. 1).

The draped operating microscope is brought into the field and is used



FIG. 2 The floor of the middle fossa. Retractor blade elevating the dura overlying the temporal lobe. The neurovascular structures have been exposed. The middle meningeal artery lies anteriorly. Behind this the great superficial petrosal nerve has been unroofed posteriorly to the geniculate ganglion. From this point further unroofing has been carried out to expose the internal auditory canal, the position of the carotid artery sigmoid sinus, and semi-circular canals.

during the remainder of the procedure. The Urban House retractor is secured to the sides of the opening, in the epidural space. Care is taken not to puncture the dura or open branches of the middle meningeal artery. The construction of the blade retractor assembly, using built-in gear trains, allows elevation of the dura without sacrificing manoeuvrability of the blade in either an anterior or posterior direction.

After elevation of the dura overlying the temporal lobe, the first landmark which the surgeon should identify is the middle meningeal artery. This is the basic landmark for all middle fossa surgery. Once the exact position of this important artery has been found, the location of other structures can be determined. Lying anterior and medial to the artery is the foramen ovale and the third division of the fifth cranial nerve. Almost directly medial, covered by a thin roof of bone, is the eustachian tube. Medial to the eustachian tube is the carotid artery, also covered by a thin layer of bone. Posterior to the middle meningeal artery is the hiatus for the greater superficial petrosal nerve. Lateral to the artery is the roof of the temporomandibular joint (Fig. 2).

After identifying the middle meningeal artery the middle fossa dura is elevated back to the petrous ridge. At the junction of the middle fossa dura and the posterior fossa dura, the superior petrosal sinus will be seen. The dura should be elevated forward following the superior petrosal sinus until the arcuate eminence comes into view. The hiatus of the greater superficial petrosal nerve can now be identified lying between the arcuate eminence and the foramen spinosum.

#### *Bone removal*

The bone above the hiatus and greater superficial petrosal nerve is usually thin. In 5% of patients it is absent posteriorly to the geniculate ganglion. This bony covering is removed with the drill using a diamond stone. The greater superficial petrosal nerve is adherent to the undersurface of the dura and should be stripped with a small dural elevator in a posterior anterior direction. This increases the anterior limit of the exposure and allows dural elevation as far anteriorly as the level of the middle meningeal artery.

The point of the retractor blade is now placed medial to the geniculate ganglion over the petrous ridge. This allows sufficient temporal lobe elevation for further bone removal with the diamond burr. After exposing the geniculate ganglion, the position of the superior semi-circular canal is determined. This is exposed with the diamond burr by removal of bone over the anterior aspect of the arcuate eminence. There may be a layer of overlying cystic bone and after removal of this, the hard, white bone of the otic capsule will be seen. The semi-circular canal appears as a thin, blue line in the thick bony matrix of the otic capsule. It should be exposed from the superior petrosal sinus laterally to the ampula. Once this has been done the posterior limit of bone removal for exposure of the internal auditory canal has been completed.

The next step in the procedure is the exposure of the lateral extent of the internal auditory canal. This is done by uncovering the labyrinthine portion of the facial nerve from the geniculate ganglion to the lateral extent of the internal auditory canal, leaving a thin, protective remnant of bone over the nerve itself.

The identification of the triangular piece of bone ("Bill's Bar") at the lateral limit of the internal auditory canal is necessary in order to separate the facial nerve anteriorly from the superior vestibular nerve posteriorly. This partition marks the actual junction of the labyrinthine portion of the facial nerve and the superior vestibular nerve. Once it has been uncovered, bone is removed over the remaining portion of the internal auditory canal and porus acousticus.

#### *The internal auditory canal*

We are now ready to open the dura overlying the internal auditory canal. An incision is made from the porus acousticus to the lateral extent of the

canal By opening the dura posteriorly a flap is obtained which is based along the anterior bony margin of the canal This serves as a protective covering for the facial nerve during the remainder of the operation

The dura is reflected and the superior vestibular nerve is identified entering the ampulla of the superior semi-circular canal This nerve is followed medially until the surface of the tumor is seen The plane between the facial nerve and the tumor can now be developed throughout the extent of the internal auditory canal because positive identification of the free nerve fibers of the superior vestibular nerve has been established prior to dissection of the tumor The surface of the tumor is usually smooth and well demarcated The tumor itself may be firm, soft or cystic Dissection of the tumor from the surrounding nerves should proceed slowly As the inferior pole of the tumor is freed, the cochlear nerve can be seen In this plane will also be seen the internal auditory artery The artery is vulnerable during most of the dissection and care must be exerted to stay close to the surface of the tumor The same technique should be used in separation of the tumor from the artery as had been used in the preservation of the nerves We must remember that the hearing function is dependent both upon a competent cochlear nerve and an intact arterial supply

After the tumor has been separated in the internal auditory canal any medial extension in the posterior fossa should be removed If a clean line of dissection has previously been developed, the tumor can usually be shelled out of its arachnoid bed in the posterior fossa without damaging surrounding structures Occasionally the lateral branch of the anterior inferior cerebellar artery will contact the surface of the tumor and a small loop of this artery may actually extend into the internal auditory canal In these situations the artery must be separated from the tumor using the same careful techniques as have been employed in the internal auditory canal

### *Closure*

After tumor removal has been completed, the operative area is inspected for small bleeding points that are controlled before proceeding to the closure The dural opening is closed using a free temporalis muscle graft This prevents postoperative spinal fluid leakage

The retractor and blade assembly are then removed The middle fossa dura is allowed to re-expand The rectangular temporal bone flap is replaced Temporalis muscle is re-approximated with interrupted suture The skin and galea are closed in one layer using interrupted wire suture

A drain is usually not necessary A dry sterile dressing is applied.

### *Postoperative care*

The patient is observed overnight in the intensive care unit Heavy antibiotic coverage in the postoperative period has not been routinely used

Ambulation is begun on the first postoperative day. Hospitalization usually runs from 7 to 10 days. The wire sutures are removed on the 14th postoperative day.

### CASE REPORTS

Five patients with intracanalicular acoustic tumors have been operated upon using the middle fossa approach. The hearing remained normal in two, was improved in two, and was lost in one. All cases retained facial function.

#### Case No. 1

*Summary* Unsteadiness two-and-a-half years prior to surgery. Total removal of acoustic tumor. Middle cranial fossa approach. Preoperative diagnosis, Menière's disease.

*History* This 60-year-old housewife began to have unsteadiness two-and-a-half years before surgery. Six months history of right hearing loss and tinnitus.

*Examination* Within normal limits except for findings related to the eighth nerve on the right. Eighth nerve (Fig. 3) SRT 50 dB PB 30°. Békésy Type IV. SISI 0%–1000 c/s, 0% 2000 c/s. Vestibular. Electronystagmography revealed 100% right canal paresis. Cerebral spinal fluid protein 34 mg %.

*X-ray* Normal temporal bones. Iophendylate studies failed to reveal presence of acoustic neuroma on the right.

*Surgery* Through a middle fossa approach, the right internal auditory canal was opened. The canal was found to be completely filled with acoustic tumor. The tumor was carefully removed and it was possible to save both the facial and cochlear nerves. The tumor arose from the superior vestibular nerve. The inferior vestibular nerve was deliberately sectioned. The tumor measured 5–6 mm. No blood transfusion was necessary.

*Postoperative course* The postoperative course was uneventful during the patient's hospitalization. She was discharged on the sixth postoperative day. There was no facial weakness.

When seen one month postoperatively the patient had experienced complete relief of unsteadiness and vertigo. The Békésy had converted from a Type IV to a Type II. The discrimination had improved from a 30% preoperative level to an 80% level at the time of this examination.

*Comment* This patient was the first in our series to undergo total removal of an acoustic tumor with preservation of both the facial and cochlear nerves. The principal reason for exploring the internal auditory canal by the middle fossa route was to relieve the patient's vertigo by selective section of the vestibular nerves with preservation of the cochlear division. When a small tumor was encountered, it was removed with preservation of the cochlear nerve. It is significant that both the plain x-rays and the iophendylate study of the posterior fossa were reported to be normal. Although a

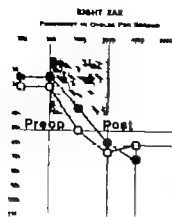


FIG 3

FIG. 3 Case 1 Pre- and postoperative pure tone audiograms.

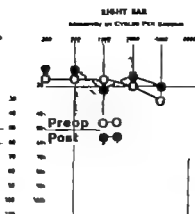


FIG 4

FIG. 4 Case 2 Pre- and postoperative pure tone audiograms.

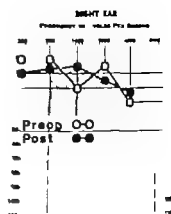


FIG 5

FIG. 5 Case 3 Pre- and postoperative pure tone audiograms.

dramatic result was obtained here, the case pointed out the need for more accurate diagnostic techniques if we were to recognize these tumors while they were still confined to the internal auditory canal.

This patient was last seen in August 1966. Her hearing has remained at an 80% discrimination level. The pure tone audiogram has remained similar to that obtained before surgery. The patient has been completely free of vertigo, unsteadiness and tinnitus since her surgery.

### Case No. 2

**Summary.** Episode of vertigo associated with hearing loss and tinnitus on the right eight months prior to surgery. Total removal of acoustic tumor, middle cranial fossa approach. Previous diagnoses included Menière's disease, vertebral basal insufficiency and multiple sclerosis.

**History.** This 42-year-old physician noted the onset of unsteadiness, hearing loss and tinnitus on the right eight months previous to surgery. The hearing loss and tinnitus remained constant. The unsteadiness was punctuated by episodes of true vertigo. One-month history of dull, aching pain within the right ear.

**Examination.** Findings were normal except for those related to the eighth nerve. Eighth nerve (Fig. 4) SRT 30 dB PB, 14% Bekésy, Type III SISI 0%–1000 c/s, 0%–2000 c/s, tone decay 80 dB–1000 95 dB–2000 Vestibular. Electronystagmography revealed 30% canal paresis on the right. Cerebral spinal fluid protein 28 mg %.

**X-ray.** Temporal bones were normal. Iophendylate study of the posterior fossa revealed a small predominantly intracanalicular tumor protruding slightly into the posterior fossa.

**Surgery.** The patient underwent a right middle fossa exploration of the right internal auditory canal. A total removal of an intracanalicular tumor



arising from the superior vestibular nerve was obtained. The facial nerve and cochlear division of the eighth cranial nerves were spared.

*Postoperative course* The patient had a moderate right facial weakness immediately postoperative. He experienced complete relief of unsteadiness and tinnitus.

Six weeks postoperatively the pure tone audiogram had returned to normal. The patient's discrimination rose to 100%. The Békésy converted to a Type I. The facial paralysis had cleared completely.

*Comment* An interesting observation by this 42-year-old physician prior to his surgery was the fading away of the loudness of the dial tone of the phone—a unique example of tone decay. Subsequent to tumor removal, the loudness of the dial tone has been maintained. This patient also demonstrates the apparent susceptibility of the cochlear division to pressure by even a small tumor in the limited confines of the internal auditory canal. Note the profound loss of discrimination ability (PB 14%), profound tone decay (9 dB-2000) and only moderate pure tone loss. After tumor removal, the restoration of these audiologic parameters to normal has been almost complete.

### Case No. 3

*Summary* One-year history of tinnitus and occasional pain in the right ear. Total removal of an intracanalicular acoustic tumor by the middle fossa approach. Excellent preservation of auditory function.

*History* This 44-year-old female noted the onset of a "tinkling sound" in the right ear one year prior to being seen. The character of the sound changed to a buzzing sensation and became continuous. Occasional deep pain in the right ear.

*Examination* Seventh nerve: Decreased sensation in the external auditory canal on the right. Eighth nerve (Fig. 5): SRT 20 dB, PB, 96%, Békésy Type II, SISL, 55%—2000 c/s, 95%—4000 c/s. Vestibular Electronystagmography revealed normal responses to caloric stimulation. Cerebral spinal fluid protein 35 mg %.

*X-ray* The entire right internal auditory canal was larger than that on the left, and there was definite evidence of a funnel-like widening of the internal auditory meatus. These findings were considered diagnostic of an acoustic neuroma on the right.

Polytome tomophenylate studies revealed failure of filling of the internal auditory canal on the right by the contrast material. There appeared to be a soft tissue mass present in the auditory meatus blocking the entrance to the internal auditory canal (Fig. 6).

*Surgery* The patient underwent a right middle fossa exploration of the internal auditory canal. An acoustic tumor was found to fill the entire canal. This was carefully separated away from the facial, cochlear and superior vestibular nerves. There was no bleeding during the dissection of the tumor. The tumor was adherent to the facial nerve and was separated



FIG. 6 Case 3. Polytom of the temporal bone to study the filling of the right internal auditory canal by contrast material. A crescent shaped defect is seen in the internal auditory canal.

away from this with some difficulty. The tumor did not extend into the posterior fossa. The estimated size was 5 mm. No blood transfusion was necessary.

*Postoperative course* The patient had moderate right facial weakness immediately following the surgery. This completely resolved within six months. She experienced some unsteadiness on rapid positional change in the first month after surgery, but this has subsided since that time. Postoperative audiologic examination six months after surgery revealed an SRT of 10 dB and a discrimination score of 100%.

*Comment* This patient has made an excellent recovery following the removal of a small acoustic tumor confined to the internal auditory canal. At this time, her hearing is normal in the operated ear and she has no facial weakness.

This patient demonstrates the importance of obtaining petrous pyramid x rays in those patients complaining of unilateral tinnitus.

#### Case No. 4

*Summary* Three month history of tinnitus and decreased hearing on the right. Occasional light headedness. Total removal of a 6 mm. acoustic tumor with preservation of the superior vestibular and facial nerves. No return of hearing subsequent to the surgery.



FIG. 7 Case 4. Polytome (ophendyl) study showing failure of filling of right internal auditory canal by contrast material. The tumor is just beginning to push out into the posterior fossa.

**History.** This 33-year-old male noted the onset of medium pitched tinnitus, accentuated by fatigue, three months prior to being seen. Associated decreased hearing on the right. One episode of itching in the ear lasting about one week. Occasional momentary light-headedness.

**Examination.** The neurologic examination was normal except for those findings related to the eighth cranial nerve. Eighth nerve SRT 45 dB PB, 40 Békésy Type II SISI, 100%–1000 c/s, 100%–2000 c/s. Vestibular Electronystagmography was normal. Cerebral spinal fluid protein 82 mg.

**X ray.** Temporal bone x-rays showed some enlargement of the right internal auditory canal with funneling at the meatus. There was slight erosion of the superior lip of the auditory meatus. These findings were considered highly suggestive of an acoustic neuroma on the right.

Polytome (ophendyl) studies showed no filling of the internal auditory canal on the right with contrast material. A crescent shaped defect was seen at the meatus. These findings were considered diagnostic of an acoustic neuroma on the right limited to the internal auditory canal (Fig. 7).

**Surgery.** The patient underwent a middle fossa exploration of the right internal auditory canal. A quote from the surgical dictation is as follows: "The dura was opened along the posterior aspect of the internal auditory canal up to the junction of the superior vestibular nerve and the facial nerve. The superior vestibular and the facial nerves were then separated by running an instrument between them starting at the bar of bone which



FIG. 6 Case 3. Petrous pyramid study showing filling of the right internal auditory canal by contrast material. A crescent shaped defect is seen at the distal end.

away from this with some difficulty. The tumor did not extend into the posterior fossa. The estimated size was 5 mm. No blood transfusion was necessary.

*Postoperative course* The patient had moderate right facial weakness immediately following the surgery. This completely resolved within six months. She experienced some unsteadiness on rapid positional change in the first month after surgery but this has subsided since that time. Postoperative audiologic examination six months after surgery revealed an SRT of 10 dB and a discrimination score of 100%.

*Comment* This patient has made an excellent recovery following the removal of a small acoustic tumor confined to the internal auditory canal. At this time, her hearing is normal in the operated ear and she has no facial weakness.

This patient demonstrates the importance of obtaining petrous pyramid x rays in those patients complaining of unilateral tinnitus.

#### Case No. 4

*Summary* Three-month history of tinnitus and decreased hearing on the right. Occasional light-headedness. Total removal of a 6 mm acoustic tumor with preservation of the superior vestibular and facial nerves. No return of hearing subsequent to the surgery.



FIG. 7 Case 4. Polytome tomophendylat study showing failure of filling of right internal auditory canal by contrast material. The tumor is just beginning to push out into the posterior fossa.

**History** This 33-year-old male noted the onset of medium pitched tinnitus, accentuated by fatigue, three months prior to being seen. Associated decreased hearing on the right. One episode of itching in the ear lasting about one week. Occasional momentary light headedness.

**Examination** The neurologic examination was normal except for those findings related to the eighth cranial nerve. Eighth nerve SRT 45 dB PB, 40% Bekésy Type II SISI 100%-1000 c/s, 100%-2000 c/s. Vestibular Electronystagmography was normal. Cerebral spinal fluid protein 85 mg. %

**X ray** Temporal bone x rays showed some enlargement of the right internal auditory canal with funneling at the meatus. There was slight erosion of the superior lip of the auditory meatus. These findings were considered highly suggestive of an acoustic neuroma on the right.

Polytome tomophendylate studies showed no filling of the internal auditory canal on the right with contrast material. A crescent shaped defect was seen at the meatus. These findings were considered diagnostic of an acoustic neuroma on the right limited to the internal auditory canal (Fig 7).

**Surgery** The patient underwent a middle fossa exploration of the right internal auditory canal. A quote from the surgical dictation is as follows: "The dura was opened along the posterior aspect of the internal auditory canal up to the junction of the superior vestibular nerve and the facial nerve. The superior vestibular and the facial nerves were then separated by running an instrument between them starting at the bar of bone which

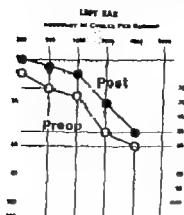


FIG. 8 Case 3 Pre- and postoperative pure tone audiograms.

separated them. The tumor was found lying in the area between all the nerves and it was carefully separated away from the facial, superior vestibular and cochlear nerves. A total removal of the tumor was accomplished. Bleeding was encountered from a branch of the internal auditory artery."

**Postoperative course** The patient made an excellent postoperative recovery. He had no facial weakness. However, hearing was not present subsequent to the surgery and six months following the operation it has not returned.

**Comment** The patient experienced good relief of the tinnitus and light headedness following the surgery. However, he did not regain any hearing function in the operated ear. One can theorize that the damage to the internal auditory artery at the time of surgery resulted in a dead ear. It must be noted also that preoperatively hearing impairment in the ear was profound both on the pure tone audiogram and the discrimination test. Probably as the neural damage increased subsequent to the enlargement of the tumor in the internal auditory canal, the chances for reversal of a hearing loss lessen.

### Case No 3

**Summary** Seven month history of tinnitus. No unsteadiness or hearing loss. Total removal of a 3 mm tumor confined entirely to the internal auditory canal with preservation of the facial, superior vestibular and cochlear nerves.

**History** This 36-year-old airline pilot noted the onset of fullness and tinnitus in his left ear seven months prior to being seen. There was no vertigo, unsteadiness or hearing loss.

**Examination** Neurologic examination was normal. Eighth nerve (Fig. 8) SRT 0 dB, PB 96%, Bekésy Type II, SISI 0%-2000, 15%-4000. Vestibular Electronystagmography was normal. Cerebral spinal fluid protein 36 mg %.

**X ray** Temporal bone x rays showed a slight enlargement of the left internal auditory canal. The meatus appeared normal.



FIG. 9 Case 4. Polytome iophendylate study showing truncated defect of the contrast column at the lateral end of the left internal auditory canal.

Iophendylate examination showed a small, crescent-shaped defect at the lateral extremity of the left internal auditory canal. This was consistent with a small mass lesion measuring not more than 5 mm (Fig 9).

**Surgery.** A middle fossa exposure of the internal auditory canal was carried out. After penning the dura, the facial and vestibular nerves were seen to be pushed upward and thinned out by an underlying mass. By separating the nerves, a small tumor was exposed in contact with the lateral end of the internal auditory canal. The tumor was mobilized and removed en bloc. It was not possible to say exactly where the tumor originated. The main trunk of the superior vestibular nerve was spared and it was assumed that the tumor originated from the inferior vestibular nerve.

**Postoperative course.** The patient had excellent hearing and facial function following the surgery. He made an uneventful recovery and was placed back on flying status six months postoperatively.

Repeat audiometric and vestibular tests were carried out four months postoperatively. These showed an SRH of 15 dB, PB 88%, Békésy Type II. Electronystagmographic testing was normal (Fig 10).

**Comment.** We anticipate a total recovery of hearing in this patient. He demonstrates a case in which we were able to eliminate, rather than accentuate a neurologic deficit. This is significant because of the patient's occupation as an airline pilot. He had been taken off flying status because of minor audiologic complaint and for this reason he was most insistent

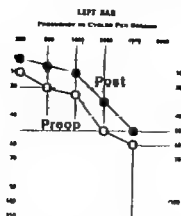


FIG. 8 Case 3 Pre- and postoperative pure tone audiograms.

separated them. The tumor was found lying in the area between all the nerves and it was carefully separated away from the facial, superior vestibular and cochlear nerves. A total removal of the tumor was accomplished. Bleeding was encountered from a branch of the internal auditory artery."

**Postoperative course** The patient made an excellent postoperative recovery. He had no facial weakness. However, hearing was not present subsequent to the surgery and six months following the operation it has not returned.

**Comment** The patient experienced good relief of the tinnitus and light-headedness following the surgery. However, he did not regain any hearing function in the operated ear. One can theorize that the damage to the internal auditory artery at the time of surgery resulted in a dead ear. It must be noted also that preoperatively hearing impairment in the ear was profound, both on the pure tone audiogram and the discrimination test. Probably as the neural damage increased subsequent to the enlargement of the tumor in the internal auditory canal, the chances for reversal of a hearing loss lessen.

#### Case No. 3

**Summary** Seven month history of tinnitus. No unsteadiness or hearing loss. Total removal of a 3 mm tumor confined entirely to the internal auditory canal with preservation of the facial, superior vestibular and cochlear nerves.

**History** This 36-year-old airline pilot noted the onset of fullness and tinnitus in his left ear seven months prior to being seen. There was no vertigo, unsteadiness or hearing loss.

**Examination** Neurologic examination was normal. Eighth nerve (Fig. 8) SRT 5 dB, PB 90%, Békésy Type II, SISI 0%-2000, 15%-4000. Vestibular Electronystagmography was normal. Cerebral spinal fluid protein 30 mg %.

**X-ray** Temporal bone x-rays showed a slight enlargement of the left internal auditory canal. The meatus appeared normal.



## PROCEDURE AND MATERIAL

An account has already been given of both the equipment (Blegvad, 1956) and the procedure (Blegvad & Terkildsen, 1956, 1957). As described by Jerger *et al.* (1959) the SISI test involves a relatively rapid and simple procedure the test technique is extremely well designed and it offers essential advantages in comparison with Lüscher's test. However the test only provides information on the ability of the patient to perceive increases in intensity of 1 dB magnitude. As it was of interest to have a more precise evaluation of the influence of contralateral masking on the sensitivity of the ear to changes in intensity the investigation was extended as mentioned, and as far as possible the psychometric function was evaluated both without and with masking in each case. Series of 20 increments of constant size within each single test-run, but varying from series to series, were presented alternatively with 0 dB masking and with 80 dB broad-band noise delivered to the contralateral ear. In the original design of the apparatus, the magnitude of the increments could be varied in steps of 0.5 dB from 5.6 dB down to 0.5 dB. It became clear at a very early stage, however, that some patients were able to hear changes in intensity even as small as 0.5 dB without any particular difficulty so an adjustment of 0.3 dB was added to the attenuator. During the investigations, it was found that in a few cases even these increments were not sufficiently difficult to detect in order to permit a study of the masking effect, since scores were almost 100% both with and without masking. However it was considered that the experimental equipment described did not allow an increment less than the one mentioned to be calibrated with reasonable accuracy. The test signal and the masking noise were delivered by insert receivers. The intensity of the continuous tone delivered was 20 dB above the threshold for the affected ear. This threshold was determined without masking, and even though masking noise might in some cases be expected to change the threshold (on account of middle-ear muscle contraction and central masking) the level of the test tone was maintained constant. For the inclusion of a patient in the material it was not a demand that the hearing of the good ear was within normal limits. Whatever the threshold in the better ear the masking was delivered at an intensity of 80 dB S.I.L. This intensity was chosen as being representative of clinical masking.

The material consists of 32 patients suffering from a predominantly unilateral hearing loss of perceptible type. Their ages varied from 25 to 4 years (mean 31 years). With regard to diagnosis, the distribution was as follows: Menière disease 21 sensorineural unknown & sudden onset of loss - 1 Lermoyez disease 1 presumably congenital loss 1. The diagnosis of Menière disease presupposed the symptoms of hearing loss, tinnitus and distur-

## DIFFERENTIAL INTENSITY SENSITIVITY AND CLINICAL MASKING

H BLEGVAD

*From the Audiological Laboratory Department of Otolaryngology University Hospital Copenhagen Denmark*

Patients with unilateral perceptive hearing loss have been examined to determine whether masking of the good ear influences the difference limen of intensity. The measurements were carried out with the aid of the SISI procedure using increments of different magnitudes, until the psychometric function was determined both without and with masking (80 dB SPL). The level of the test tone was maintained constant (20 dB above the threshold determined without masking). At 1000 and 4000 cps, the contralateral noise resulted in a significant improvement in the intensity discrimination, while no change occurred at 250 cps. With a few exceptions, the masking had no significance for the result of the topognostic SISI test using 1 dB increments.

Two decades have elapsed since measurement of the difference limen of intensity (DLI) was introduced as a test in diagnostic audiometry (Lüscher & Zwislöcki, 1948). As mentioned in a previous paper (Blegvad, 1966) a number of investigations have been made on the diagnostic value of Lüscher's test. Especially since the description of the SISI test by Jerger *et al* (1959) the investigation of the sensitivity of the ear to changes in intensity has found wide application in the topical diagnosis of hearing loss. Several studies are also now available on the reliability of the SISI test and on a range of variables which are of significance for the results of the test. A review of these studies has been provided by Harford (1967). Difference limen measurements have been employed both in the case of unilateral and bilateral hearing loss. Although not always reported in the literature it is probable that masking of the good ear has been a regular procedure in the examination of patients with unilateral hearing loss. As reported in a preliminary communication (Blegvad & Terkildsen 1966) we have observed that in some patients with unilateral perceptive hearing loss, the results of the investigation are influenced by the use of masking noise. As far as we are aware this factor has not been taken into consideration previously and the aim of the present investigations is to evaluate this problem in greater detail.

This work was supported by a grant from Statens Almindelige Videnskabsfond

be carried out.<sup>1</sup> Finally and most important, the investigation was so time-consuming that in ambulatory patients it could be carried out at only one frequency. In general, between 1 hour and 1½ hours was devoted to each frequency. The total of complete investigations at each separate frequency was as follows: 250 cps,  $n=21$ ; 1000 cps,  $n=23$ ; 4000 cps,  $n=20$ .

### RESULTS

The results were analysed in the same way as in the investigation of the influence of contralateral masking on the SISI test in normal hearing subjects. In each psychometric function, four values of intensity increment were chosen which had given the nearest scores around 50%. In some cases, only three values could be used, and in a few cases only two values, as even the least increment had given a score near 50%. In these cases the 0% point of the curve was calculated by linear extrapolation. The slope of the psychometric functions and the 50% point of the functions were determined by means of the area method. The slope was calculated as the increase in score (in %) for an increase in increment magnitude of 1 decibel. The 50% point indicates the increment magnitude at which on the average the subject will reply on half the number of occasions, and is usually taken to indicate the DLI. The  $t$ -test was used to determine whether the mean difference between the values obtained without and with masking differed significantly from 0.

Table 1 gives the results for the slope of the functions. As stated, masking gave no significant shift in slope. Table 2 shows the results for the 50% point. Both at 1000 and 4000 cps, the masking resulted in a significant shift towards a lower value. The SISI scores for 1 dB increments were classified in the usual three categories: 0-1% negative, 20-50% questionable, 60-100% positive. The distribution of the results, without and with contralateral masking (the latter shown in *italics*) was as follows: 250 cps negative 15, 14 questionable, 6 positive; 1000 cps negative 7, 5 questionable, 11 positive; 4000 cps negative 9, questionable 6, 7 positive.

TABLE 1. Mean slope of psychometric functions for intensity variations (in dB) and with masking of the good ear. Results from patients with unilateral perceptible deafness.

Slope: Mean of individual slopes determined with SISI method expressed in % per 1 dB. The changes with masking were of significant level at the 5% level.

Masking level (dB SFL)	250 cps ( <i>n</i> = 21)		1000 cps ( <i>n</i> = 23)		4000 cps ( <i>n</i> = 20)	
	Slope	Change	Slope	Change	Slope	Change
0	60.8		101.9		97.5	
40	68.6	7.9	89.6	-12.3	104.6	7.1

<sup>1</sup> In the course of the investigation we encountered this problem in four patients and only at 1000 cps. It is evident that the omission of these patients with particularly acute bilateral influences on the DLI at this frequency as well as the distribution of SISI scores.

ance of equilibrium, whereas it was not regarded as necessary criteria that the patient should suffer from vertigo in acute attacks or that his acoustic symptoms should fluctuate in step with any possible attack. No patient was included in the material if the result of Fowler's binaural balance test and/or Metz (1952) test was negative. With regard to the audiometric configuration of the hearing loss, the distribution was as follows (classified according to Johnson & House 1964) High tone 6 low tone 7 flat loss 17 trough shape 2.

The investigations were made at 250 1000 and 4000 cps. The threshold of the frequencies investigated was within the limits 35-75 dB (re ISO 1964 norm). The sequence in which the frequencies were investigated varied, just as the different increment magnitudes were presented in varying order. With regard to the reliability of the SISI test Jerger *et al* (1959) quote the results of investigations on a single patient who was examined 8 times over a period of 10 months by three different investigators. At 1000 cps the scores varied from 0 to 5% at 4000 cps the maximum variations were from 85 to 95%. This stability was characterized as typical. Jerger subsequently (1962) investigated test-re-test reliability by requesting 27 patients with perceptive hearing loss to attend for re-examination. The greatest test-retest difference (12.3%) was found at 4000 cps. The coefficient of reliability ( $r$ ) was found to be low at 250 cps (0.10) but the determination is claimed to have suffered from a quite restricted range as large SISI scores are rare at this frequency. At 1000 and 4000 cps there was reasonable consistency ( $r=0.72$  and  $0.88$  respectively). It is our experience that even within the same sitting the results of the investigations can show considerable variations, variations which can be ascribed neither to training nor to mental fatigue. With the same stimulus, the score can even fluctuate between positive and negative. Since in some patients a contralateral masking effect seemed to be present in an extremely discreet form, a single measurement was not considered sufficiently positive evaluation. Determining the psychometric function both with and without masking, the measurement was repeated using those increments which had given scores around 50%. I.e. those variations in intensity which were at the boundary of audibility. In most cases, a single re-test was not considered sufficient and test runs with those increments which represented the steep portion of the psychometric curve were repeated 2-3 times. On the average, 4 repeat test runs were carried out at 250 cps, 5 at 1000 cps and 6 at 4000 cps. At 250 cps, the psychometric function was generally less steep than at higher frequencies, and the course of the curve was determined at a greater number of measuring points. For this reason fewer re-tests were made at this frequency despite its low coefficient of reliability. The mean values were employed in the statistical analysis. Only a proportion of the patients could be examined at all three frequencies. In a number of cases the threshold lay outside the limits chosen and in a few cases the sensitivity to the variations in intensity was so great that as stated above the investigation could not

be carried out.<sup>1</sup> Finally and most important, the investigation was so time-consuming that in ambulatory patients it could be carried out at only one frequency. In general, between 1 hour and  $1\frac{1}{2}$  hours was devoted to each frequency. The total of complete investigations at each separate frequency was as follows: 250 cps,  $n=21$ ; 1000 cps,  $n=23$ ; 4000 cps,  $n=20$ .

## RESULTS

The results were analysed in the same way as in the investigation of the influence of contralateral masking on the SISI test in normal hearing subjects. In each psychometric function, four values of intensity increment were chosen which had given the nearest scores around 50%. In some cases, only three values could be used, and in a few cases only two values, as even the least increment had given a score near 50%. In these cases the 0% point of the curve was calculated by linear extrapolation. The slope of the psychometric functions and the 50% point of the functions were determined by means of the area method. The slope was calculated as the increase in score (in %) for an increase in increment magnitude of 1 decibel. The 50% point indicates the increment magnitude at which on the average the subject will reply on half the number of occasions, and is usually taken to indicate the DLI. The *t*-test was used to determine whether the mean difference between the values obtained without and with masking differed significantly from 0.

Table 1 gives the results for the slope of the functions. As stated, masking gave no significant shift in slope. Table 2 shows the results for the 50% point. Both at 1000 and 4000 cps, the masking resulted in a significant shift towards a lower value. The SISI scores for 1 dB increments were classified in the usual three categories: 0-15% negative, 20-55% questionable, 60-100% positive. The distribution of the results, without and with contralateral masking (the latter shown in italics) was as follows: 250 cps negative 14, 14; questionable 3, 6; positive 1, 1. 1000 cps negative 7, 5; questionable 4, 6; positive 12, 12. 4000 cps negative 9, 7; questionable 3, 7; positive

TABLE 1. Mean slope of psychometric functions for intensity variations in quiet and with masking of the good ear. Results from patients with unilateral perceptible deafness.

Slope: Mean of individual slopes determined with SISI method expressed in % per 1 dB. The brackets with masking were not significant at the 5% level.

Masking level (dB SPL)	250 cps (n=21)		1000 cps (n=23)		4000 cps (n=20)	
	Slope	Change	Slope	Change	Slope	Change
0	66.3		101.0		97.5	
50	68.6	1.9	97.6	3.5	104.6	7.1

In the course of the investigation we encountered this problem only at 1000 cps. It is evident that the masking of these patients with particularly acute differential sensitivity influences our mean DLI at this frequency as well as the distribution of SISI scores.

TABLE 2 *Mean DLI (dB) of patients with unilateral perceptive deafness in quiet and with masking of the good ear*

DLI: Averaged 50% points of psychometric functions for intensity variations as determined with SISI method. Asterisk indicate significance level of the changes caused by masking ( \* and \* for  $p < 1\%$  and  $0.1\%$  respectively)

Masking level (dB SPL)	250 cps (n=21)		1000 cps (n=23)		4000 cps (n=20)	
	DLI	Change	DLI	Change	DLI	Change
0	1.77		1.26		1.35	
80	1.68	-0.08	1.08	-0.18	1.13	-0.22

6. Thus, the masking influenced to topognostic result of the current clinical test in only a very few cases. Even though the mean change in DLI as a result of the contralateral masking was only slight numerically the change in score was at times striking, as shown below

At 1000 cps, the score in patient K. K. in four test-runs with 0.5 dB increments was 5, 35, 40 and 35% when the contralateral ear was masked without masking, the values in all four cases were 0%. At the same frequency the score for J. H. with 1 dB increments and masking was 65, 0, 50% while without masking it was 0% on each occasion. H. K. scored 20, 10, 20% for 1.5 dB without masking with masking, 80, 100, 93%. With the same stimulus, K. O. H. scored without and with masking 5, 5, 15% and 65, 45, 45%. For a 2 dB stimulus, the corresponding results in this patient were 35, 50% and 65, 80%.

At 4000 cps, E. S. in five test runs without masking responded to 4.5, 10, 35, 50 and 30% of the 0.5 dB stimuli presented with masking to 80, 40, 75, 80 and 60%. With 1 dB stimuli, D. P. at the same frequencies scored 5, 5, 20% against 50, 55, 60%. K. S. scored 43, 30, 43, 50% against 90, 95, 90, 95%. At 1.5 dB in the latter patient, the results were 20, 5, 35% without masking and 50, 45, 70% with contralateral masking. Finally in E. B. without masking the score for 2.0 dB was 10, 20, 30, 10% against 65, 95, 95, 80% with 80 dB masking.

## DISCUSSION

The investigations have shown that the difference limen of intensity in patients with unilateral perceptive hearing loss is improved significantly at 1000 and 4000 cps, when the good ear is masked. No change occurs at 250 cps. Even though there may have been crosshearing in some of the investigations without masking, this cannot explain the effect of the masking. The results for 250 cps tell against the possibility that middle-ear muscle contraction may play a significant role. In our investigations of the influence of contralateral masking on the SISI test in normal hearing subjects, we found that the masking noise improved the intensity discrimination at 1000 and 4000 cps, while a significant impairment in discrimination took place at 250 cps. It would seem reasonable to explain the difference found between normal and pathological ears at 250 cps as being due to the difference in

stimulation intensity in both series of investigations the test was performed at 20 dB above the individual threshold, so that the absolute intensity at which the cochlea was stimulated was greater for the pathological group. Owens (1965) has pointed out that high SISI scores in cochlear hearing loss can only be expected provided the hearing loss is greater than 30 dB (re ASA 1951 standard). Swisher *et al* (1968) have shown that in sensorineural deafness SISI scores (determined 20 dB above the subjective threshold) increase systematically with increasing HL (hearing level: the physical intensity of a tone given in relation to the normal threshold for the tone in question). They also found no distinction between the difference limen of intensity in normal hearing subjects and in patients with perceptible loss of hearing, when the basis for comparison was the HL at which the measurement was made. These results are in accordance with earlier experience using the Lüscher test. In Lüscher's department, while measurements were originally made 40 dB above the patient's threshold (Lüscher & Zwislöcki 1948) the procedure subsequently followed (cf. for example, Lüscher 1955) was to make the determination at a fixed intensity (80 dB HL). At this intensity the difference limen of intensity in patients with a cochlear type hearing loss (Lüscher: in hearing loss with complete recruitment) is said to be equal to that of normal hearing subjects. The observations suggest that the sound pressure level entering the cochlea is decisive for the magnitude of the difference limen. Our results show that the relationships involving the difference limen are more complicated than the above mentioned investigations might suggest. At 1000 and 4000 cps, a reduction in DLI was found on noise stimulation of the contralateral ear although the intensity of the test tone was maintained constant all the time and in normal hearing subjects we found contralateral masking capable of eliciting positive SISI scores at 20 dB hearing level (4000 cps). When the results were plotted for the present material (admittedly limited) no striking difference was found between the masking effect at moderate hearing loss and at severe hearing loss (> 60 dB) but the relationship between the intensity of the test tone and the masking undoubtedly deserves further study.

It has long been known that masking of one ear can change the threshold for pure tones in the other ear. According to Lidén *et al* (1959) this effect, usually designated the *central masking effect*, can amount to 15 dB, even in patients with inactive middle-ear muscles. According to these authors the change is usually of minor importance in clinical audiometry. Ward (1965) has stated with regard to clinical masking, perhaps central effects and reflex attenuation will usually be the second-order effects they have been thought to be. Our investigation have shown that what is affected is not merely the threshold measured by standard audiometry: also the results of Bekésy audiometry (Bjergvad, 1968) and the difference limen of intensity are affected by contralateral masking. Our studies suggest that stimulation of the non-test ear causes changes in the audiological tests which are of both theoretical and practical clinical significance.

TABLE 2 *Mean DLI (dB) of patients with unilateral perceptive deafness in quiet and with masking of the good ear*

DLI: Averaged 50% points of psychometric functions to intensity variations as determined with SISI method. Asterisk indicate significance level of the changes caused by masking ( and for  $p < 1\%$  and  $0.1\%$  respectively)

Masking level (dB SPL)	250 cps (n=21)		1000 cps (n=23)		4000 cps (n=20)	
	DLI	Change	DLI	Change	DLI	Change
0	1.77		1.26		1.35	
80	1.68	-0.08	1.08	-0.18	1.13	-0.22

§ 6 Thus, the masking influenced to topognostic result of the current clinical test in only a very few cases. Even though the mean change in DLI as a result of the contralateral masking was only slight numerically, the change in score was at times striking, as shown below.

At 1000 cps, the score in patient K. K. in four test runs with 0.5 dB increments was 5, 35, 40 and 35% when the contralateral ear was masked without masking, the values in all four cases were 0%. At the same frequency the score for V. H. with 1 dB increments and masking was 65, 0, 50% while without masking it was 0% on each occasion. H. K. scored 20, 10, 20% for 1.5 dB without masking with masking, 80, 100, 95%. With the same stimulus, L. O. H. scored without and with masking, 5, 5, 15% and 65, 45, 45%. For a 2 dB stimulus, the corresponding results in this patient were 35, 50% and 65, 80%.

At 4000 cps, E. S. in five test runs without masking responded to 45, 10, 35, 50 and 30% of the 0.5 dB stimuli presented with masking to 80, 40, 75, 80 and 60%. With 1 dB stimuli D. P. at the same frequencies scored 5, 5, 20% against 50, 55, 60%. K. S. scored 45, 30, 45, 55% against 90, 95, 90, 95%. At 1.5 dB in the latter patient, the results were 20, 5, 35% without masking and 55, 45, 70% with contralateral masking. Finally in E. B. without masking, the score for 2.0 dB was 15, 25, 30, 10% against 65, 95, 95, 80% with 80 dB masking.

## DISCUSSION

The investigations have shown that the difference limen of intensity in patients with unilateral perceptive hearing loss is improved significantly at 1000 and 4000 cps, when the good ear is masked. No change occurs at 250 cps. Even though there may have been crosshearing in some of the investigations without masking, this cannot explain the effect of the masking. The results for 250 cps tell against the possibility that middle-ear muscle contraction may play a significant role. In our investigations of the influence of contralateral masking on the SISI test in normal hearing subjects, we found that the masking noise improved the intensity discrimination at 1000 and 4000 cps, while a significant impairment in discrimination took place at 250 cps. It would seem reasonable to explain the difference found between normal and pathological ears at 250 cps as being due to the difference in



## CLINICAL APPLICATION OF INTENSITY DIFFERENCE LIMEN

F. HARRERT, I. M. YOUNG and B. G. WEISS

From the Department of Otolaryngology Jefferson Medical College Philadelphia Penn., U.S.A

SISI tests were administered at various sound pressure levels in normal and pathologic subjects. When the inner ear receives an audible signal of 60 dB SPL or greater a positive SISI score will occur in both normal and sensorineural hearing losses except in those with abnormal adaptation. A negative SISI score in the absence of a conductive barrier occurs only in abnormally adapting ears and is probably indicative of supra-threshold adaptation. Our data indicate that recruiting ears and normal ears perceive intensity increments of equal size to equivalent SISI. In abnormally adapting ears, as Békésy separation increases, the increment tends to become larger than normal. Continuous tone Békésy threshold measured at the bottom of the first spike increase directly with starting intensity and inversely with attenuation rate. Variations of the SISI test and Békésy audiometry are proposed.

The intensity difference limen (DLI) has been measured by many methods. The Lüscher & Zwislocki (1948, 1949) and Denes & Naunton (1950) clinical tests have been replaced by the widely used SISI test (Jerger *et al.* 1959). The amplitude of Békésy tracings has also been considered an indication of the DLI (Békésy 1947). Closely related to tests of the DL are the phenomena of recruitment, defined as an abnormal growth of loudness, and adaptation, described as a reduction in loudness during stimulation. Recruitment is classically measured by the alternate binaural loudness balance test (Fowler 1928) or the monaural loudness balance test (Ruger 1936). Adaptation may be measured at threshold by the tone decay test (Schubert, 1944; Carhart, 1957) and by Békésy audiometry. Threshold drift and wide separation between pulsed and steady tone Békésy tracings are examples of threshold adaptation. Suprathreshold adaptation may be measured by a simultaneous loudness balance procedure described by Hood (1950) and by a delayed loudness balance test reported by Wood (1930).

### PART I

The size of the difference limen for intensity in normal ears varies among subjects and depends on the method of testing. Nine years after the intro-

Presented at the combined meeting of Sections on Otolaryngology College of Physicians of Philadelphia, and the New York Academy of Medicine with the Philadelphia Laryngological Society at Philadelphia, Pennsylvania on March 20, 1968.

## ZUSAMMENFASSUNG

Man hat bei Patienten mit einseitiger perzeptiver Schwerhörigkeit untersucht, ob die Maskierung des guten Ohres Einfluss auf die Intensitätsunterschiedsschwelle haben werde. Die Messungen wurde mittels der SISI Prozedur mit Intensitätsänderungen verschiedener Grösse unternommen bis die psychometrische Funktion teils ohne teils mit Maskierung (80 dB SPL) festgestellt wurde. Das Niveau des Test Tons wurde konstant gehalten (20 dB über die Schwelle wie ohne Maskierung gemessen). Bei 1000 und 4000 Hz verursachte das kontralaterale Geräusch eine signifikante Besserung der Intensitätsdiskrimination, bei 250 Hz geschah keine Änderung. Mit wenigen Ausnahmen war die Maskierung ohne Bedeutung für das Ergebnis der topognostischen SISI Prüfung mit 1 dB Intensitätsänderungen.

## REFERENCES

- BLEGVAD, H. 1966: The SISI-test in normal listeners. In investigation with non-commercial equipment. *Acta Otolaryng* (Stockh.) 69 201
- 1968: Békésy audiometry and clinical masking. *Acta Otolaryng* (Stockh.) 66 229
- BLEGVAD, H. and TENKILDAER, K. 1966: Békésy audiometry SISI test and contralateral masking. Preliminary report. *Acta Otolaryng* (Stockh.) 62, 453
- 1967: Contralateral masking and the SISI test in normal listeners. *Acta Otolaryng* (Stockh.) 63 537
- HARFORD, E. R. 1967: Clinical application and significance of the SISI test. In *Sensorineural hearing processes and disorders* (ed. A. B. Graham) p. 223. (Henry Ford Hospital International Symposium) J & A Churchill, London.
- JERGER, J. 1962: Comparative evaluation of some auditory measures. *J Speech Hearing Res* 5 3.
- JERGER, J., SHEDD, J. L., and HARFORD, E. 1959: On the detection of extremely small changes in sound intensity. *Arch. Otolaryng* (Chic.) 69 200
- JOHNSON, E. W. and HOUSS, W. F. 1964: Auditory findings in 53 cases of acoustic neuroma. *Arch. Otolaryng* (Chic.) 80 667
- LIDÉN, G., NILSSON, G. and ANDERSSON, H. 1959: Masking in clinical audiometry. *Acta Otolaryng* (Stockh.) 50 125
- LÜSCHER, E. 1955: Unsere Bestimmung der Intensitätsmodulation reiner Töne deren Anwertung und deren psychologische Leistungsfähigkeit. *Acta Otolaryng* (Stockh.) 4 402
- LÜSCHER, E. and ZWISLOCKI, J. 1948: Eine einheitliche Methode zur monauralen Bestimmung des Recruitmentphänomens (Lautstärkengleich). *Pract. Otolaryng* (Basel) 10 521
- METZ, O. 1952: Threshold of reflex contraction of muscles of middle ear and recruitment of loudness. *Arch. Otolaryng* (Chic.) 5 536
- OWENS, E. 1965: The SISI test and VIIIth nerve versus cochlear involvement. *J Speech Hearing Dis* 30 232
- SWANBERG, L., P. STERNBERG, M. M. and DOMANI, D. G. 1966: The effect of hearing level and normal variability on sensitivity to intensity change. *J Aud Res* 6 249
- WARD, W. D. 1963: Auditory fatigue and masking. In *Modern developments in audiology* (ed. J. Jerger) p. 232. Academic Press, New York and London.

B. Blegvad M.D.  
60 Vingard II  
2900 Hillside, Minneapolis

Received May 27 1968

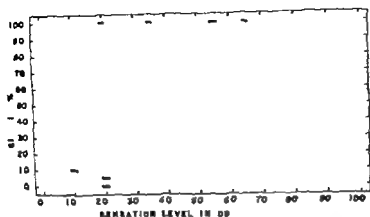


FIG. 2. SISI scores of the same 17 cochlear lesions with thresholds lower than 60 dB SPL plotted on the basis of sensation level.

to near 100% above 50 dB at all frequencies tested. In naive normal subjects, this change occurs at 60 dB (Fig. 1). In two groups clinically diagnosed as having either Menière's syndrome or abrupt drop hearing loss, the change also occurs at 60 dB as in naive normals (Young & Harbert, 1967). It is universally accepted that these cases have cochlear lesions, yet they do not differ from normal ears without hearing loss when intensity is plotted in SPL. When the same data of the Menière's group is plotted as a function of SL, all of these cases showed SISI scores between 80 and 100% at sensation levels both below and above 20 dB. Presbycusis and congenital sensori-neural hearing loss show the same abrupt change at 60 dB as normals and admittedly cochlear lesions (Young & Harbert, 1967). Because there were few subjects with hearing levels below 60 dB SPL in this series, another group of cochlear lesions with minimal hearing loss was tested. Fig. 2 plotted in terms of SPL shows that this group of 17 behaved similar to the



FIG. 4. SISI scores of 17 cases of conductive hearing losses as function of SPL without subtracting the conductive barrier.

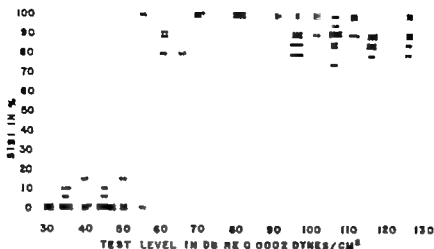


FIG. 1 SISI scores of 33 naive normal listeners tested at various frequencies.

duction of the SISI test, investigators and clinicians are still not in agreement on either the best size of the increment or the significance of the test.

The SISI test records the percentage of 1 dB increments heard out of 20 trials. The increments are superimposed on a continuous tone delivered at 20 dB sensation level (SL). Normal ears and those with a significant conductive hearing loss or abnormal adaptation fail to hear most, if not all of the 20 increments. Ability to hear a high percentage of the presentations is considered indicative of a "cochlear lesion" (Yantis & Decker 1964; Owens, 1965).

By employing sensation level the inner ear is subjected to variable intensities depending on the degree and type of hearing loss. If it can be shown that a pathologic ear behaves as a normal ear when subjected to signals at equivalent intensities, the diagnostic significance of the test is in doubt.

When percentage score is plotted against SPL in trained normal listeners, there is a sharp change from near zero % at intensities below 50 dB SPL

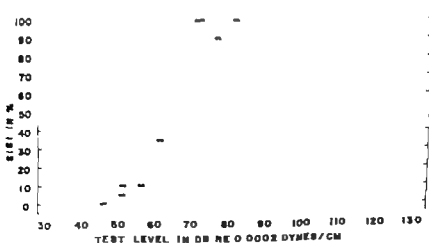


FIG. 2 SISI scores of 17 cochlear lesioned subjects with minimal hearing losses (thresholds lower than 60 dB SPL at frequencies from 250 Hz through 8000 Hz) as a function of SPL.

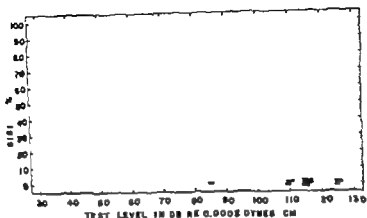


FIG. 8. SISI scores of 13 abnormally adapting ears tested at intensities below the established Békésy constant tone threshold.

The SISI test is usually administered with a high signal to noise ratio. Tonndorf *et al* (193 ) and Sherrick (1939) have shown that as the S/N ratio increases the DLI becomes smaller and that sensation level is not important. These investigators sought the size of the DL by varying the S/N ratio and concluded that for a given S/N ratio, the DL was constant and independent of the SL. In this study the increment was constant and the per cent score was plotted as a function of S/N ratio. If noise is deliberately added to a signal at 60, 80, and 100 dB SPL, there is a sudden change in the SISI score for a 1 dB increment when the S/N ratio is reduced to +5 dB or below. This change from near 100% to near 0% is as dramatic as the change from 30-00 dB in the absence of noise (Fig. 7). When the increment is increased above 1 dB, detectability in the presence of noise is enhanced. For a 2 dB increment the signal to noise ratio is -5 dB, for a 3 dB increment it is -15 dB.

In a study (Weiss *et al* 1967) on the minimum size of the increment that is recognizable in normal and pathological ears, the following findings are of interest:

1. In 63 naive normal ears, the size of the increment varied between 0.5 and 1.5 dB when a 230 msec increment with a rise and fall time of 20 msec was presented near the end of a 5 second, 80 dB SPL, pure tone signal.
2. In 41 ears with recruitment the intensity increment fell within this normal range. Our data also did not sustain the notion that recruiting ears can detect smaller increments than normal ears at equivalent SPL by this method.
3. In abnormally adapting ears, increment data suggested that as separation between continuous and interrupted Békésy tracings increases, the increment tends to become larger than normal.

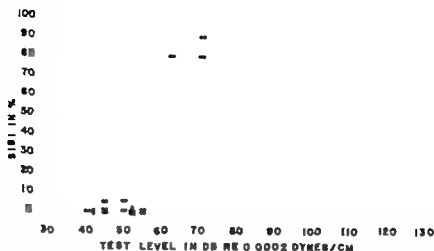


FIG. 5 SISI scores of the same 17 conductively deafened ears as a function of SPL after the conductive barrier was subtracted from the testing levels.

naïve normal group. Again when the same data are plotted as a function of SL, it is apparent that more than half show low SISI scores at 40 dB SL (Fig. 3). All of the positive scores were in subjects receiving the signal at 55–65 dB SPL. Low SISI scores occurred when subjects received the signal at 55 dB SPL or below. This is in accord with the findings of Harford (1965) that low SISI scores accompany mild losses in some cases of cochlear pathology. This emphasizes that SPL rather than SL is the important parameter in determining the score value.

When percentage scores in conductively deafened ears are plotted on the basis of SPL, there are a large number of negative scores even at high intensities (Fig. 4). When these same data are plotted after subtracting the conductive barrier, these ears also show an abrupt change in SISI scores at 60 dB as in all of the above (Fig. 5). Only abnormally adapting ears show negative SISI scores at SPL both above and below 60 dB (Fig. 6).

These findings indicate that a positive SISI score in a naïve listener means the inner ear is receiving a signal at an intensity of 60 dB SPL or above and is behaving as a normal ear at this intensity. Since trained normal listeners notice this change at 50 dB SPL, it appears likely that subjects who undergo repeated testing or are acute observers may also respond with high scores at this level and above. This is in keeping with Riesz's finding (1928) that in normal ears the differential sensitivity for frequencies 32–8192 Hz varied between 0.2 and 0.5 dB from 60 dB to 130 dB SPL by his method of testing. Swisher (1966) and Swisher *et al.* (1966) also showed that normal and non-adapting sensorineural impaired ears discriminated a signal of 1 dB or less equally well at equivalent SPL.

If the SISI score is low (negative) when the inner ear receives a signal at 60 dB SPL or above, it probably indicates an abnormally adapting ear and this behavior can be equated with abnormal tone decay, significant separation between pulsed and steady tone, Békésy tracings, etc.

approached alternately from no sound to sound and sound to no sound, the single number representing threshold was considered to be a line connecting the midpoints of spikes. On the basis of thresholds for various attenuation rates and relation to conventional audiometry we consider the bottoms of spikes the best single measure of threshold (Harbert & Young, 1962, 1966). If the threshold is approached from sound to no sound the threshold of inaudibility is invariably better than the threshold of audibility determined by proceeding from no sound to sound. This difference represents the amplitude of Békésy spikes. For some reason, if the ear is hearing a decreasing signal it is able to continue hearing in an area where it never hears when the sound is increasing from an inaudible level. The more rapid the rate of change, the better the threshold of inaudibility as measured by the amplitude of spikes. If the amplitude is known for a given attenuation rate, the amplitude for other attenuation rates can be predicted. Doubling the attenuation rate increases the amplitude by about 62% (Harbert & Young, 1966). Reduced amplitude therefore represents a defect in the ability to continue to hear a sound decreasing from threshold. We consider this a manifestation of rapid adaptation which takes place in milliseconds.

The disorder which causes reduced amplitude of continuous tone Békésy tracing does not cause a corresponding reduction of pulsed tone amplitude. There appears to be no close correlation between amplitude width and recruitment as measured by the alternate binaural loudness balance test. The concept of threshold recruitment implies that the reduced amplitude represents a "sharper" threshold than normal. In other words, the pathological ear performs better than a normal one.

For pulsed tones, the threshold of the bottom of the first spike is the same whether threshold is approached from below or above except in certain markedly adapting ears. For continuous tones, there is a threshold increase of 1.5 dB for every 20 dB increase of suprathreshold starting intensity in normal ears. There is a similar 1.5 dB increase in threshold for each halving of the attenuation rate in both normal ears and those with cochlear lesions. For a group of cochlear lesions each 20 dB increase in suprathreshold starting intensity causes a mean elevation of threshold of about 3 dB. In markedly adapting ears, the effect may be much more. Frequency does not seem to be a factor (Harbert & Young, 1968).

These findings may be the basis for another clinical test for abnormal adaptation.

1. The threshold for a fixed frequency continuous tone is approached three times from below and the mean recorded.
2. The threshold is similarly approached from the limit of the machine and its mean recorded.
3. The difference between these would be a measure of adaptation to be compared with the normal amount.
4. In ears with marked abnormal adaptation there may be a similar but lesser effect for pulsed tones.

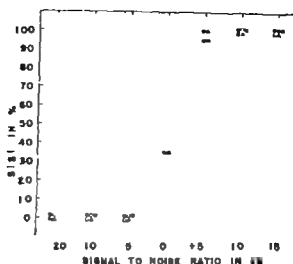


Fig. 7 SISI scores for 1000 Hz at 100 dB SPL in five trained normal listeners as a function of S/N ratio.

In order to minimize adaptation to the carrier signal in the SISI test, it is suggested that the test be modified as follows:

- 1 After a practice period to ensure recognition of the increment and understanding of the test, interrupt the signal after each presentation by briefly (1 second) depressing the interrupter switch. Record score for each presentation. The selector switch to eliminate the increment should be randomly activated after several positive responses have been obtained to eliminate a false positive response.

- 2 The increment should be set at 1.5 dB and the signal presented at 70 dB re ISO (1964) for frequencies 500 to 8000 Hz and 55 dB re ISO for 250 Hz. When hearing loss approaches these levels, the signal should be increased to an intensity at least 10 dB above threshold. The conductive barrier in dB should also be added. In abnormally adapting ears, the test level must be above the stabilized Békésy threshold for valid results.

- 3 A positive score means that the ear is responding as a normal ear. If a negative score is obtained the size of the increment may be increased until it is heard to measure the degree of impairment of the DLI.

## PART II

The Békésy audiometer is a device to record the threshold of a tone that is changing in intensity at a constant rate usually by a method of limits technique. Instructions are to press a button as soon as the tone is heard and to keep it depressed until it disappears. While the button is depressed the signal decreases. On the tracing, every rising limb represents a period of hearing and every descending limb a period of no hearing. This has been described as "variability about the threshold" (Hirsch *et al.* 1954) but this variability is not random. Because the threshold was considered to be



- 1943: Effects of varying attenuation rate and starting intensity on Wiley threshold. *J Acoust Soc Am* 13, 732.
- HARROLD, E. R., 1943: Clinical application and significance of the SISI test Presented at the International Symposium, "Sensorineural hearing processes and disorders" Detroit, Michigan.
- HINCK, L. J., PALA, T. and GOODMAN, A., 1934: Difference Limen and recruitment. *Arch Otolaryng* (Chic.) 69 823.
- HOOG, J. D. 1930: Studies in auditory fatigue and adaptation. *Acta Otolaryng* (Stockh.) Suppl. 32.
- JENSEN, J., SUNDØ, J. and H. RØRD, E., 1930: On the detection of extremely small changes in sound intensity. *Arch Otolaryng* (Chic.) 69 200.
- LISCHKE, E., and ZWISLOCKI, J. 1948: Eine einfache Methode zur monauralen Bestimmung des Lautstärkenunterschieds. *Arch Ohren Nas. Kehlkopfheilk* 133, 322.
- 1949: A simple method for indirect monaural determination of the recruitment phenomenon (difference limen in intensity in different types of deafness). *Acta Otolaryng* (Stockh.) Suppl. 78, 136.
- OWEN, E., 1945: The SISI test and VIIIth nerve crosses cochlear implantment. *J Speech Hear Dis*, 10 252.
- RADEK, E. V., 1936: Differences in loudness response of the normal and hard-of-hearing ear at intensity levels slightly above the threshold. *Ann. Otol.* 5, 1929.
- RADEK, R. R. 1928: Differential intensity sensitivity of the ear for pure tones. *Physiol. Rev* 31 849.
- SCHUBERT, K., 1944: Hörschwelle und Hördauer. *Arch. Ohrenheilk* 31 19.
- SEIDENFELD, C. E., JR., 1936: Effect of background noise on the auditory intensity difference limen. *J Acoust Soc. Am* 31 229.
- SUNDØ, L. P. 1946: Response to intensity change in cochlear pathology. *Laryngoscope* 76, 1766.
- SUNDØ, L. P., STEPHENS, M. M., and DORRANCE, D. G., 1946: The effects of hearing level and normal variability on sensitivity to intensity change. *J Aud. Res.*, 6, 249.
- TROOP, J., BROOKS, F. and WASHBURN, D., 1945: Auditory difference limen of intensity in normal-hearing subjects. Report No. 33-31 USAF School of Aviation Medicine.
- WARR, B. G., HARRIST, F. and WILKINS, C. R., 1947: Speech threshold intensity discrimination. Presented to the American Speech and Hearing Association Convention, Chicago, Illinois (Nov).
- WOOD, A. C., 1930: A quantitative account of the course of auditory fatigue. Unpublished master's thesis, University of Virginia.
- YITTA, P. A., and DECKER, R. L., 1944: On the short increment sensitivity index (SISI test). *J Speech Hear Dis*, 29 231.
- YOUNG, I. M., and HARRIST, F. 1947: Significance of the SISI test. *J Aud. Res.*, 7 343.

Dept. of Otolaryngology Jefferson  
Medical College Philadelphia,  
Penn., U.S.A.

Received April 30 1968

## CONCLUSIONS

- 1 A positive SISI score when the inner ear perceives a signal of 60 dB SPL or more, probably has no significance since it occurs in both normal ears and sensorineural deafened ears with a wide variety of diagnoses.
- 2 A negative SISI score when the inner ear receives an audible stimulus at 60 dB SPL and above, is an indication of abnormal intensity discrimination and is probably indicative of suprathreshold adaptation.
- 3 Reduced amplitude of Békésy continuous tone tracings probably is a measure of rapid adaptation.
- 4 Variations of SISI and Békésy tests are proposed.

## ZUSAMMENFASSUNG

An normalhörenden Personen und pathologischen Fällen wurde die Änderung der Quote des SISI Tests mit wachsendem Schalldruckpegel aufgenommen. Wird dem Ohr ein für den Patienten hörbares Signal mit einem Schalldruckpegel von 60 dB oder mehr zugeleitet, so ist die SISI Test-Quote sowohl bei normalhörenden Personen wie bei neurogenen (sensorineural) Hörverlustfällen positiv jedoch unter Ausnahme der neurogenen Fälle mit abnormaler Adaptation. Ein negativer Ausfall des SISI Tests findet sich sofern keine zusätzliche Leitungsfehlerkomponente vorliegt, nur in Fällen mit abnormaler Adaptation. Aus den vorliegenden Daten ist des weiteren zu entnehmen dass bei gleichem Schalldruckpegel die gerade wahrnehmbare Intensitätszunahme für Ohren mit Recruitment und für normalhörende Ohren von gleicher Grösse ist. In Fällen mit abnormaler Adaptation dagegen bei denen die Békésy Audiogramme mit kontinuierlicher und pulsierender Tonfolge in zunehmendem Masse divergieren fallen die gerade wahrnehmbaren Intensitätsänderungen eher grösser als normal aus. Weiterhin wurde die Abhängigkeit des Beginns der Schwellenzeichnung der Békésy Aufnahme mit kontinuierlicher Tongabe (bei konstanter Frequenz) von der Einsatzintensität der Tongabe und der Geschwindigkeit der automatischen Intensitätsänderung untersucht. Im Zusammenhang damit werden gewisse Modifikationen in der Durchführung des SISI Tests und hinsichtlich der Einsatzintensität der Tongabe bei der Békésy Audiogrammaufnahme vorgeschlagen.

## REFERENCES

- BÉKÉSY G. 1947. A new audiometer. *Acta Otolaryng* (Stockh.) 35: 411.  
 GARNANT R. 1957. Clinical determination of abnormal auditory adaptation. *J. Otolaryng* (Chic.) 65: 32.  
 DEXTER, P. and NATHAN, R. F. 1940. Threshold detection of auditory recruitment. *J. Laryng* 64: 37.  
 FOWLER, E. P. 1948. Method of measuring normal and abnormal recruitment. *Otolaryng* (Chic.) 56: 151.  
 HARBERT F. and YOUNG, I. M. 1962. Threshold auditory adaptation. *J. Aud. R.* 2: 229.  
 — 1966. Amplitude of Békésy tracing with differential threshold. *J. Acoust. Soc. Am.* 39: 914.

Nerve innervation of the bronchial tract, such as excitatory fibers from the vagus and inhibitory fibers from the sympathetic to the bronchial muscle, is mainly concluded on the basis of physiological evidence. Although some attempts (Larsell, 1922; Honjin, 1936; Fisher, 1965) were made to observe the nerve innervation of the respiratory tract by staining with silver or methylene blue, the adrenergic nerves could not be differentiated from other types of nerves. Concerning the study of the distribution of the adrenergic nerves by histochemical demonstration of the monoamine oxidase, Fujiwara *et al.* (1966) showed that this enzyme tended to distribute to the effector cells.

The fluorescent histochemical demonstration of the endogenous *n*-noradrenaline developed by Falck (1962) seemed to be the best histological device available to study the adrenergic innervation of the tissues. According to the simplified modification of Falck's method, Terayama & Soda (1967) demonstrated the noradrenaline-fluorescent fibers only in the vessels of the submucous and muscular layers of the esophagus. Dahlstrom *et al.* (1968) observed the specific fluorescent fibers innervating the bronchial muscles in cats and suggested that some part of the innervation derived from the pathogenesis of the bronchiectasia.

In the present experiments the presence of the specific fluorescences of noradrenaline produced by the exposure of the freeze-dried tissue sections to formaldehyde in the esophagus and bronchial tract of the rabbit were histochemically observed.

## MATERIAL AND METHODS

Twenty male albino rabbits, each weighing about 2 kg, were used. The animals were sacrificed by the exsanguination from the cut ends of both common carotid arteries, and immediately thereafter the whole length of the esophagus and both lungs with the trachea were extirpated. The esophagus was divided into three parts, the upper (cervical), middle (thoracic) and lower (thoraco-lumbar) thirds. The respiratory tract was also divided into three parts, the cervical trachea, tracheal bifurcation, and lung. Along with the rabbits, some guinea-pig and rats were also used.

The fluorescent histochemical procedures of the esophagus and respiratory tract were performed according to the original method of Falck (1962).

The freeze-dried preparations were treated with formaldehyde gas at 80°C for 1 hr, then infiltrated *in vacuo* with paraffin at 60°C for 10 min. Sections (4–8  $\mu$ ) were placed on non-fluorescent slides and deparaffinized by careful addition of xylene. The microscopical observation of the tissue section was performed by means of a Zeiss fluorescence microscope equipped with an HBO 200 high pressure mercury lamp, a Schott BG 12 (3 mm) excitation filter, dark field condenser, and a Schott OG 4 filter (1 mm) in the tube.

## THE ADRENERGIC INNERVATION IN THE ESOPHAGUS AND RESPIRATORY TRACT OF THE RABBIT

T. NISHIMURA and T. TAKASU

*From the Department of Otolaryngology Nagoya City University Medical School  
Nagoya Japan*

The mode of innervation of the adrenergic nerve fibers in the esophagus and respiratory tract of the rabbit was observed fluoro-histochemically. The noradrenaline fluorescent fibers in the esophagus were found to distribute in the submucosa lamina muscularis mucosa muscle layers, and around the myenteric ganglion cell. The dense distribution of the fibers around the myenteric plexus, arteries, and arterioles showed the net structures with varicose terminals, indicating active regulation of the structures. The fluorescent fibers in the trachea were found to distribute in the submucosa and muscular layers by penetrating the fibro-elastic layer and membranous portion of the submucosa. The distribution of the fluorescent fibers in the lung was relatively abundant around the pulmonary arteries, but the smooth muscle of the bronchiole exhibited a few fluorescent fibers, which were completely absent from the alveoles.

A peristaltic wave appears in the upper end of the esophagus associated with each swallowing effort. This wave consists of an esophageal constriction caused by the contraction of the external bundles of striated muscle at the site of the wave and relaxation beyond. The striated muscle is directly innervated by the vagus nerves without the intervention of any local ganglion cells. In the smooth muscle portion of the lower third of the esophagus, however, there is an extensive local plexus including nerve cells and their branches similar to the myenteric plexus in other parts of the gastrointestinal tract (Greving 1919). Further in animals such as dogs and rabbits, in which the entire esophagus is made up of external striated muscle, high cervical vagotomy results in a permanent inability to swallow. Based on the observation that the thoracic and cervical sympathectomy in dogs produced an increased sticking of the food on the mucous membrane of the esophagus, Kure & Okinaka (1956) suggested adrenergic participation with the esophageal motility. The longitudinal internal muscularis mucosa of the esophagus is a smooth muscle and it increases in thickness toward the cardia of the stomach. In spite of several histological attempts (Kadanoff & Spassova, 1959; Temesrékusi, 1959; Liven, 1961) no clear-cut evidence to support the regulatory mode of the sympathetic innervation on the esophageal function has been presented hitherto in available literature.



FIG. 2. The middle portion in the esophagus of a normal rabbit. The specific fluorescent fibers (+) are much more abundant in the external muscle layer (EL) than in the medial (ML) and internal layers. The specific fluorescent fibers in the adventitia of an artery (AT) are observed between the external and medial layers. 126.

muscle, showed a fairly dense distribution of the specific fluorescent fibers laterally (Fig. 2). On the other hand, the fluorescent fibers were much less in the circular muscles and especially in the smooth muscle of the lamina muscularis mucosa. Auerbach's ganglion cells, found in the myenteric space between the longitudinal and circular muscle layers, were surrounded by a dense network of fluorescent varicose fibers (Fig. 3). These fibers showed occasionally a continuous connection with the fluorescent fibers penetrating into the external longitudinal and internal circular muscles. Auerbach's ganglion cells were non fluorescent. The arteries with the intermediary caliber observed frequently in the longitudinal muscle and adventitia, also showed dense distribution of the fluorescent fibers, mainly in their adventitial layers (Fig. 4).

No significant difference in distribution of the specific fluorescent fibers was found between the upper and middle thirds of the esophagus. On the other hand, the fluorescent fibers and Auerbach's nerve cells, with the surrounding dense net of the fluorescent fibers in the lower third of the esophagus, were found to be increased toward the cardia of the stomach together with an increase in thickness of the inner longitudinal muscularis mucosa.

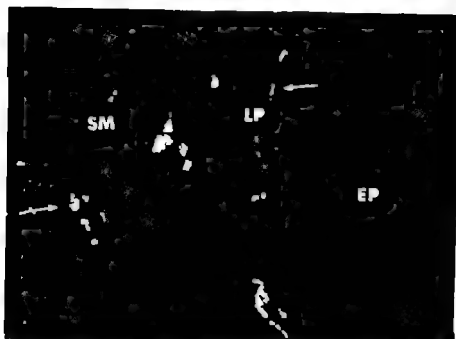


FIG. 1 The upper portion in the esophagus of a normal rabbit. The specific fluorescent fibers (†) in the lamina propria mucosa (LP) and the submucosa (SM) are abundant, but are absent from the epithelium (EP)  $\times 160$

The endogenous noradrenaline in the tissues exhibited a green or yellowish green fluorescence. In addition the specific fluorescence of noradrenaline was confirmed by comparison of the adjacent tissue section without treatment with paraformaldehyde with the tissue sections taken from the animals previously treated intraperitoneally with 1 mg/kg of reserpine 18 hours before.

## RESULTS

### *Distribution of the adrenergic nerves in the esophagus*

The rabbit esophagus consists of the following five layers: the mucous epithelium, lamina propria mucosa, submucosa, circular and longitudinal muscles, and tunica adventitia. The superficial cornification layer of the epithelium and the stratified squamous epithelium exhibited sometimes a non-specific fluorescence. However, a specific fluorescence of noradrenaline did not appear. A considerably dense distribution of the noradrenaline-fluorescent fibers was observed in the lamina propria mucosa and the submucosa. As shown in Fig. 1 the fluorescent fibers in these layers were abundant, especially around the arteries and arterioles. However, no ganglion cell similar to the Meissner's cell found in the intestinal tract was observed in the submucosa. Also, no specific fluorescent fiber was found around the submucosa glands. The muscular layers exhibited also the presence of the specific fluorescent fibers. However, the distribution of the fluorescent fibers differed considerably in number according to the respective muscle layers. The longitudinal muscularis externa, though it consisted of a striated



FIG. 5. The circular portion of the trachea of normal rabbit. The dense distribution of the specific fluorescent fibers (f) is observed in the adventitia of the arteries (AT) in the submucosa (SM). The fluorescence in the lamina propria (LP) are non-specific. 128.

The numerous granular and yellowish fluorescences present in the lamina propria, similar to those found in the medial and intimal layers of the big artery were non specific and seemed to derive from the elastic fibers. The serous and mucous glands in the lamina propria were not surrounded by the specific fluorescent fibers. The arteries and arterioles frequently observed in the submucosa exhibited the dense distribution of the fluorescent fibers mainly in the adventitia (Fig. 5). In addition, the specific fluorescent fibers penetrating the fibro-elastic layer and the smooth muscle of the membranous portion of the submucosa were also observed, sometimes in



FIG. 6. The arborescent fibers with specific fluorescence in the adventitia of the pulmonary artery. The fibers form typical monopole ground plexus. 328.



FIG. 3 The lower portion of the esophagus of a normal rabbit. The Auerbach plexus is found to be surrounded by a dense network of fluorescent varicose fibers. 320

#### *Distribution of the adrenergic nerves in the respiratory tract*

The rabbit trachea consisted histologically of the ciliated pseudostratified columnar epithelium, lamina propria rich in elastic fibers and poor in the serous and mucous glands, submucosa and cartilage. In the posterior portion of the trachea the glands extended through the muscular layer to the submucosa. The epithelial cell did not exhibit the specific fluorescence.



FIG. 4 The lower portion of the esophagus of a normal rabbit. The specific fluorescent fibers are observed: the distal end of an artery (AT) and the elastic lamellae (EL) in the medial muscular layer (ML) and the Auerbach plexus (AP). The ganglion cells are non-fluorescent. 160





FIG. 5. The cervical portion of the trachea of a normal rabbit. The dense distribution of the specific fluorescent fibers (+) is observed in the adventitia of the arteries (AT) in the submucosa (SM). The fluorescence in the lamina propria (LP) are non-specific. 123

The numerous granular and yellowish fluorescences present in the lamina propria, similar to those found in the medial and intimal layers of the big artery were non specific and seemed to derive from the elastic fibers. The serous and mucous glands in the lamina propria were not surrounded by the specific fluorescent fibers. The arteries and arterioles frequently observed in the submucosa exhibited the dense distribution of the fluorescent fibers mainly in the adventitia (Fig. 5). In addition, the specific fluorescent fibers penetrating the fibro-elastic layer and the smooth muscle of the membranous portion of the submucosa were also observed, sometimes in



FIG. 6. The viscous fibers emitting specific fluorescence in the adventitia of the pulmonary artery. The fibers form a typical autonomic ground plexus. 120

the portion of the trachea. The distribution of the fluorescent fibers did not markedly differ between the cervical trachea and the bifurcation.

Although the pattern of the distribution of the fluorescent fibers in the bronchus was similar to that of the trachea, the distribution decreased in density progressively toward the periphery together with a decreased caliber. The smooth muscle of the bronchiole exhibited a few fluorescent fibers, which were completely absent from the alveoles. However, the pulmonary vessels and especially the pulmonary arteries showed the dense distribution of the fluorescent fibers in the adventitia, immediately adjacent to the smooth muscle of the media. These fibers with the varicose structure formed an autonomic ground plexus (Fig. 6) as described by Falek (1964) and Norberg (1964) and some of the fibers seemed to invade the media. On the other hand, the pulmonary veins as well as the peribronchial arteries and veins showed decreased distribution of the fluorescent fibers.

### DISCUSSION

The greenish yellow fluorescences found in the esophagus and respiratory tract of the rabbit were likely to represent the endogenous noradrenaline in the adrenergic nerve fibers because of the following evidence: (1) The fluorescence presented a fiber-like structure with a varicose terminal in the fiber course. (2) The physiological presence of the endogenous noradrenaline in the esophagus and respiratory tract of the rabbit was also confirmed biochemically by the present authors (1967). The mean levels of the amine in the whole esophagus, trachea and bronchus, and lung were 0.08, 0.20, and 0.03  $\mu\text{g/g}$  respectively. The estimated values seemed to coincide roughly with the distribution of the specific fluorescence found in these tissues.

As described above, the vagus nerve has an excitatory regulation on the spontaneous and swallowing movement of the esophagus. It was not obvious whether the mechanism of relaxation was derived from the adrenergic nerve or not. However, Kuro & Okinaka (1956) showed the increased sticking of the food on the esophageal mucous membrane in the sympathectomized dog. In the esophagus of the rabbit the noradrenaline-fluorescent fibers proved to distribute in the internal, medial, and external muscle layers, and around the arteries and arterioles in the submucosa and adventitia as well as around the myenteric nerve cell. The myenteric plexus in the esophagus of the guinea pig was also confirmed to be distributed with fluorescent fibers, in contrast to the findings of Terayama & Soda (1967). The distribution of the adrenergic nerve fibers around the myenteric plexus in the intestine was first presented by Norberg (1964) fluorohistochemically. Since the myenteric nerve cell confirmed to show a high activity of the intracellular cholinesterase, did not exhibit a specific fluorescence, this cell was concluded to be purely cholinergic in nature. However, the dense distribution of the fluorescent fibers with the varicose terminals and net structures around the cell indicates the adrenergic innervation, and consequently

the adrenergic regulation, of the cell function. The downward increase in thickness of the longitudinal muscularis mucosa in the esophagus seemed to correlate with the similar downward increase in the number of myenteric nerve cells. Many fluorescent fibers were also found in the lamina propria and submucosa around and apart from the blood vessels.

The mutually antagonistic innervation of the respiratory tract by the autonomic nerves has been established. However no confirming histological study on the adrenergic innervation has been presented. In accordance with the description by Terayama & Soda (1967) the fluorescent adrenergic nerves in the trachea of the guinea pig extended to the submucosal layer by penetrating the fibro-elastic layer and the smooth muscles of the membranous portion and distributed to the smooth muscles and arteries. The fluorescent distribution of the adrenergic nerve fibers to the bronchial muscles was already shown by Dahlström *et al* (1966) in the cat. The relatively less developed serous and mucinous glands in the trachea and bronchus of the rabbit did not show the distribution of the adrenergic nerve fibers. The pulmonary artery showed the most dense distribution of the fluorescent fibers, which formed the autonomic ground plexus in the adventitia. Some of the fibers were found to invade the smooth muscle of the media. According to Norberg & Hamberger (1964) and Ohgushi (1967) such distribution of the fluorescent fibers in the smooth muscle of the media was rarely observed in the peripheral artery. The pulmonary veins and peribronchial arteries exhibited a relatively smaller distribution of the fluorescent fibers, but the distribution was significantly more intense than that found in the bronchial muscle. Bruner & Schmidt (1947) and Murao (1966) have shown that the blood flow of the bronchial vessels is increased by adrenergic stimuli and decreased by vagal stimulation. Therefore, adrenergic stimuli can affect more profoundly the bronchial circulation system, enriched by the adrenergic supply than the bronchial muscle poorly supplied by the same nerves.

## ZUSAMMENFASSUNG

Es wurde eine Fluoreszenzhistochemische Untersuchung über den Innervationsmodus adrenergischer Nervenfasern der Speiseröhre und der Luftwege bei Kaninchen durchgeführt. Die adrenergisch fluoreszierenden Nervenfasern der Speiseröhre ließen sich in der Schleimhaut, Lamina muscularis und in allen drei Muskelschichten sowie um die myenterischen Ganglienzellen nachweisen. Nervenfasern, die sich insbesondere um die myenterischen Nervenplexus, Arterien und Arterien nicht befinden, zeigten die rosenkranzartige Struktur eines Terminalretikulums, wobei eine aktive regulierende Funktion dieser Struktur angedeutet werden konnte. In der Lufttröhre war zu erkennen, dass sich die Fluoreszenz der Fasern, die elastischen Schleimhaut und Pars membranacea der Submukosa durchsetzend, in der Submukosa und den Muskelschichten verteilten. In der Lunge zeigte sich vor allem um die A. pulmonalis eine relativ reichliche Innervierung obengrenzter Fasern, wohingegen sie in der glatten Muskulatur der Bronchiolen kaum zu finden waren und in den Alveolen vollständig fehlten.

## ACKNOWLEDGMENT

The authors express thanks to Prof. K. Shimamoto and Assist. Prof. M. Fujiwara of the Pharmacology Department Kyoto University for their continued interest and guidance in this investigation.

## REFERENCES

- BRUNNEN H. D. and SCHMIDT C. F. 1947 Blood flow in the bronchial artery of the anesthetized dog. *Amer J Physiol* 148: 648.
- CARLSSON A., FALCK, B., and HILLARP N. A. 1962: Cellular localization of brain monoamines. *Acta Physiol Scand* 36 Suppl. 196.
- DAHLSTRÖM A., FUXE, K., HÖKFLYT T., and NORDBERG, K. A., 1966. Adrenergic innervation of the bronchial muscle of the rat. *Acta Physiol Scand* 66: 507.
- FALCK, B. 1962. Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta Physiol Scand* 36 Suppl. 197.
- 1964. Cellular localization of monoamines. *Progress in Brain Research* vol. 31. *Biogenic Amines* (ed. H. E. Himwich and W. A. Himwich) Elsevier Publ. Co. Amsterdam.
- FALCK, B., HILLARP N. A., THIEME, G. and TORP A. 1962. Fluorescence of catecholamine and related compounds condensed with formaldehyde. *J Histochem. Cytochem* 10: 348.
- FISCHER, A. W. F. 1963. The intrinsic innervation of the pulmonary vessels. *Acta Anat* 60: 481.
- FUJIMURA, M., TANAKA, C., HIRAYAMA, H. and OKADA, T. 1966. Cytological localization of norepinephrine monoamine oxidase and acetylcholinesterase in salivary glands of dog. *J Histochem. Cytochem* 14: 485.
- GREVING, N. 1919: Die Innervation der Speiseröhre. *Zf angew Anat & hist. Histochem* 101: 327.
- HOMMA, R. 1956. On the innervation of the lung of the mouse with special reference to the structure of the peripheral efferent nervous system. *J Comp Neurol* 105: 587.
- KADAKOFF, D. and SPASSOW, J. 1959: Über die Formen der Ganglienzellen in den intramuralen Plexus der Speiseröhre beim Menschen. *Acta Neurol (Basel)* 20: 118.
- KURO, K., and OKADA, K. S. 1956. *Anatomical Syllabus* 6th ed. Rinsen Shoten, Tokyo.
- LANSBELL, O. 1922. The ganglionic innervation of the mammalian lung and pleura pleural plexus. *J Comp Neurol* 35: 97.
- LIEN, A. V. 1961: Über die Innervation der Speiseröhre bei Weibchen und Männchen. *Z Mikroskop. Anat. Forsch* 67: 441.
- MURAO, H. 1965. Vascular regulation of the bronchial circulation in Japan. *Jap Circul J* 29: 8.
- NISHIMURA, T. In preparation.
- NORDBERG, K. A. 1964. Adrenergic innervation of the heart. I. Well studied by fluorescence microscopy. *Int J Pharmacol* 3: 379.
- NORDBERG, K. A. and HENRIKSSON, B. 1964. The sympathetic adrenergic innervation of the heart. *Physiol Sci* 63: Suppl. 238.
- OHGAKI, N. 196. Personal communication.
- TAKASU, T. and D. 1959. Vergleichende histologische Untersuchungen an den Nerven im menschlichen Speiseröhre. *Z Mikroskop. Anat. Forsch* 62: 661.
- TERAYAMA, Y. and SODA, T. 1967. Fluorescence microscopy and distribution of adrenergic fibers in the esophagus, trachea and bronchi of guinea pigs. *Otolaryngol Fukuoka* 13: 10.

Dept. of Otolaryngology  
Yagyu City University of Medicine  
Yagyu City, Japan

Received June 6, 1968

*Acta oto-laryng* 67

## THE BACTERIOLOGY OF THE RESPIRATORY TRACT IN VARIOUS PATHOLOGICAL CONDITIONS

Z. HRAJICA, F. HOSKOVIČ and I. BARIĆ

*From the Otorhinolaryngological Clinic and the Pediatric Clinic Medical Faculty  
University of Zagreb Zagreb Yugoslavia*

A total of 111 samples of diseases of the middle ear nose paranasal sinuses, and specific and non-specific pulmonary diseases were examined bacteriologically. On the basis of our examinations, we offer the following conclusions. The swab of the ear in acute inflammation of the middle ear has less diagnostic value if the secretion is not pronounced. In these cases we find the bacterial flora sensitive to penicillin. In chronic inflammation the bacterial flora is mixed and antibiotics with the largest spectrum give the best result. The bacterial flora in different pathological conditions of the tracheobronchial tree are not only a combination of several antibiotics can be successful. The bacteriological examination of the paranasal sinuses must be with clear inflammatory secretion or washing with bouillon, because washing with physiological solution can hide the bacteriological picture with *Haemophilus influenzae*. Parallel bacteriological examinations of the nose and paranasal sinuses did not give the same bacteriological findings, which means that the bacteriological finding of the nose cannot be a bacteriological indication of infection of the paranasal sinuses.

During the last 20 years the use of antibiotics has brought about a series of changes in both the clinical and pathological findings in acute and chronic infection. We have witnessed the discovery of a large number of antibiotics with a limited or broad antibacterial activity. However their uncontrolled and wide off-schematic use has sometimes caused their value to be discredited. Furthermore it has caused changes in the bacterial flora as a causative agent of various pathological conditions in the region of the ear nose throat, and bronchi. This state of affairs has induced us to study in detail the bacteriological examination in our profession in order to give some directions for antibiotic treatment of infection of the upper and lower respiratory tract. It is certain that infection of the upper respiratory tract can involve the lower respiratory pathways and vice versa, but can do so only if the resistance of the respiratory mucosa is reduced. The flora in the respiratory tract can be of different composition even in areas located as close to each other as the nose and paranasal sinuses. We wanted to give

This work has been supported by the Yugoslav Foundation for the Furtherance of Research Work.

special attention to the paranasal sinuses because it has become an established practice in our profession to consider a negative result of puncture as indicative of an absence of infection even if radiological findings are positive. This assumption is absolutely false. Bacteriological examination is of special importance in such cases. While acute otitis in most instances poses no problem with antibiotic treatment because it is generally susceptible to penicillin, chronic otitis and conditions resulting from radical trepanation still remain problems for the otologist. We are aware of the fact that the general protective forces of the organism play a certain role in the effectiveness of many antibiotics, in addition to their specific effect—this has to be taken account in the interpretation of the disproportion in the difference of the *in vitro* and *in vivo* effect.

A total of 516 samples of diseases of the middle ear, nose and maxillary sinus and in specific and non specific diseases of the lung were submitted to bacteriological study.

## RESULTS

### The ear

A total of 138 samples of swabs recovered from the cavity, antrum, or mastoid cells during surgery were examined bacteriologically (see Table 1). Among these 138 examined samples there were 32 which were sterile. Potentially pathogenic flora was found in 86 samples and saprophytic flora in 45 samples. According to the diagnosis, we examined 31 cases of otitis media acuta, 82 cases of otitis media chronica, 11 swabs from radical trepanation, 11 samples taken in the course of surgical intervention, and one sample each of otitis bullosa haemorrhagica, blue drum, and otitis externa. In acute otitis media without perforation and in blue drum the discharge

TABLE 1

ST—St. rile, PP—potentially pathogenic, SA—saprophyte, P—pneumococcus, SH—streptococcus haemolyticus, STP—*Staphylococcus pyogenes aureus*, EC—*Escherichia coli*, PR—proteus, PY—*penicillium monas pyocyanea*, KL—*Klebsiella*, C—*candida*, STA—*staphylococcus aureus*, S\—*streptococcus viridans*, SC—*saprophytic cocci*, D—*diphtheroid*.

	Potentially pathogenic flora												Saprophytic flora			
	No.	ST	PP	SA	P	SH	STP	EC	PR	P\	KL	C	STA	S\	SC	D
1. Otitis med. chr.	31	21	4	8	3	1	0	0	0	0	0	0	2	2	2	2
2. Otitis med. chr.	82	6	72	25	2	4	22	3	25	10	1	2	12	1	8	3
3. Antritis per.	8	0	4	4	2	0	1	0	0	1	0	0	3	0	1	0
4. Mastoiditis oper.	3	0	3	0	0	0	2	1	0	0	0	0	0	0	0	0
5. St. post oper. rad.	11	3	3	6	0	0	0	0	0	1	0	2	3	0	0	0
6. Otitis bul. hem.	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7. Blue drum	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8. Otitis externa	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Total	138	32	86	45	6	5	27	4	23	12	2	4	21	3	11	6

was recovered through the ear drum by aspiration with a needle fixed to a syringe.

If we analyse the bacterial flora and the results of bacteriological examinations in acute otitis media, we see that potentially pathogenic flora was found in only 4 out of 31 cases, i.e. pneumococcus in 3 cases and streptococcus haemolyticus in one. Saprophytic bacterial flora either alone or in combination with potentially pathogenic flora was found in 8 cases. Twenty-one samples remained sterile.

The findings of flora and the ratio of potentially pathogenic flora to saprophytic flora differed considerably in cases of chronic otitis media. Potentially pathogenic flora was found in 72 out of 82 examined cases: bacteria from the proteus group (25) staphylococcus pyogenes aureus (22) pseudomonas aeruginosa (10) streptococcus haemolyticus (4) and pneumococcus (2). Saprophytic bacterial flora was found in 23 instances, either alone or mixed with pathogenic flora. Only 9 swabs in this group remained sterile.

Samples recovered during surgical intervention were all positive: out of a total of 11 samples there were 7 positive for potentially pathogenic flora and 4 for saprophytic flora. Among the samples of potentially pathogenic flora there were 3 containing staphylococcus pyogenes aureus, 2 containing pneumococcus, and one each containing *Escherichia coli* and pseudomonas aeruginosa.

In conditions resulting from radical surgical intervention, 3 samples out of a total of 11 swabs were sterile, potentially pathogenic flora was isolated from 3 swabs, and saprophytic flora from 6. Staphylococcus pyogenes aureus was found twice and pseudomonas pyocyanea once in the samples of potentially pathogenic flora.

Samples recovered in blue drum and otitis bullosa haemorrhagica remained sterile: saprophytes only were found in otitis externa.

### Branchi

*Technique of recovering swab* Sterile glass tubes slightly thinner and longer than a bronchoscope served as protection from pollution on the way to the bronchi for the sterile cotton swab fixed to a wire.

A total of 120 samples of bronchial discharge from the same number of patients were examined bacteriologically (see Table 2). Of this number 87 were suffering from pulmonary disease due to mycobacterium of tuberculosis, i.e. 38 with the diagnosis atelectasis, 26 with infiltratio pulm., 16 with adenitis hilli pec., 4 with pneumonia caecosa, and 3 with the millaris pulm. Twenty-two of a total of 87 samples remained sterile: potentially pathogenic flora and 112 saprophytic causative agents were found in 57. Potentially pathogenic flora was isolated as follows: streptococcus haemolyticus (22) pneumococcus (16) staphylococcus pyogenes aureus (14) haemophilus influenzae (7) *E. coli* (2) and candida (1). In almost all cases these causative agents were isolated concurrently with abundant sapro-

TABLE 2

HI = *Haemophilus influenzae* EN = enterococcus, SN = saprophytic bacteria. See Table 1 for other abbreviations.

	Potentially pathogenic flora											Saprophytic flora				
	No	ST	PP	SA	P	SH	STP	EC	PR	G	HI	EN	STA	SV	SN	SC
1 Atelectasis	38	13	17	57	8	7	4	5	1	1	2	0	12	19	23	4
2. Infiltratio pulm. tbc.	26	7	27	27	3	9	8	0	1	0	2	0	0	9	12	6
3. Adenitis hill tbc	16	1	11	28	3	4	1	1	0	0	1	0	3	8	8	7
4. Pneumonia caseosa	4	0	3	1	1	1	1	0	0	0	1	0	0	1	0	0
5. Tbc. miliaris	3	1	3	1	1	1	0	0	0	0	1	0	0	1	1	0
6. Pneumonia and st. post	12	1	7	8	1	1	1	1	1	0	1	1	0	4	2	0
7. Bronchiectasia	10	0	14	12	1	4	2	2	0	0	5	0	0	4	4	4
8. Bronchitis	5	3	0	2	0	0	0	0	0	0	0	0	0	1	1	0
9. Others	6	0	8	7	1	3	3	0	0	0	1	0	0	3	1	0
Total	10	26	86	141	19	30	20	0	3	1	14	1	15	50	51	21
Total specific	87	22	57	112	16	22	14	6	2	1	7	0	15	38	42	18
Total non-specific	33	4	29	29	3	8	6	3	1	0	7	1	0	12	8	4

phytic flora. In the group with a total of 33 cases of non specific diseases there were 12 cases with the diagnosis of bronchopneumonia, pneumonia, or st post pneumoniam, 10 cases of bronchiectasiae and 5 of bronchitis. The remaining 6 cases in this group included diagnoses such as abscessus pulmonum, mediastinitis, tumor pulm., etc. The most frequently isolated causative agent among the potentially pathogenic agents in this group was streptococcus haemolyticus, as it was in the group preceding it. This agent was isolated in 8 cases. It was followed by *Haemophilus influenzae* in 7 cases (5 of which were bronchiectasiae), staphylococcus pyogenes aureus in 6 cases, *E. coli* in 3 cases, and enterococcus and proteus in one case each. Findings of saprophytic flora were far more frequently present than they were in the group of patients suffering from specific pulmonary diseases.

### Sinuses

Total number of patients examined	110
Total number of samples examined	246
1 Puncture only	10
2 Lavage with saline only	1
3 Lavage with bouillon	19
4 Nasal swab + lav. bouillon + saline	68

**Technique of collecting secretion from sinus.** Two puncture needles the liquid is injected through one and the contents of the sinus are collected through the other.

A total of 246 samples from 110 patients suffering from acute or chronic



TABLE 3

See Tables 1 and 2 for abbreviations.

No.	Potentially pathogenic flora											Saprophytic flora		
	ST	PP	SA	P	SH	STP	EC	PR	G	HI		STA	SV	SC
Puncture only	18	9	5	4	0	0	3	0	0	0	3	1	1	2
Saline lavage only	7	3	2	2	0	1	0	0	0	0	1	0	0	2
Bouillon lavage only	19	3	12	5	1	4	4	1	0	2	1	4	1	0
Total	42	17	20	11	1	5	6	1	0	3	5	5	2	4
Nasal swab	68	35	16	18	3	3	4	1	1	2	4	9	4	5
Bouillon lavage	68	34	14	18	3	2	2	4	1	2	1	7	0	11
Saline in age	68	32	4	13	1	0	0	2	1	0	0	3	0	10

sinusitis maxillaris were studied (see Table 3). Bacteriological examinations were performed on punctates from the maxillary sinus—either samples obtained by puncture or material obtained by lavage with sterile saline (S.S.) or sterile glucose bouillon. Sixteen samples of punctate were collected, 7 samples of lavage with sterile saline and 10 samples of lavage with sterile glucose bouillon. In 68 patients 3 samples were taken concurrently (1) nasal swabs, (2) lavage with sterile glucose bouillon, and (3) lavage with sterile saline. By comparing these techniques of sample collection, we endeavored to discover the best technique of recovering samples from the maxillary sinus.

Among the 16 cases in which the punctate only was taken, the samples collected from 9 patients remained sterile, and from the samples of 5 patients potentially pathogenic causative agents were isolated twice *staphylococcus pyogenes aureus* and three times *H. influenzae*. Saprophytic flora was found in 4 cases.

In the group of 16 patients from whom samples were recovered by means of sinus lavage with saline there were 5 sterile samples, 2 of which contained potentially pathogenic flora, i.e. *streptococcus haemolyticus* and *H. influenzae*.

In the group of 16 patients from whom samples were recovered by means of lavage with sterile bouillon the number of sterile samples was as low as 5. Potentially pathogenic flora was found in 13 and saprophytic flora in 5. Among the potentially pathogenic flora found there were *streptococcus haemolyticus* and *staphylococcus pyogenes aureus* 4 times each, proteus twice and pneumococcus, *H. influenzae* and *E. coli* once each.

As already mentioned, in 68 patients 3 examinations, i.e. nasal swabs, lavage with glucose bouillon, and lavage with saline were carried out simultaneously. The samples taken from the nasal swab and by lavage with sterile glucose bouillon equaled each other in their number of sterile samples—35 and 34, respectively. The samples containing potentially patho-

genic flora also were almost equal in number i.e. their ratio was 16 : 14. Of the 68 samples taken by means of sterile saline lavage 52 were sterile. potentially pathogenic flora was found in 4 samples only and saprophytic flora in 13 samples.

Comparison of the results obtained in samples recovered by means of nasal swabs and sinus lavage with sterile bouillon showed that the same causative agents were not always isolated. That is to say while *H. influenzae* was isolated from nasal swabs in 4 cases and staphylococcus pyogenes aureus in 21 cases, the samples obtained by means of lavage with sterile bouillon fairly often contained the coliform group of *E. coli* and proteus. Only once did we succeed in isolating *H. influenzae*—hence we can only assume that contamination of the medium had probably occurred in the course of the intervention. The remaining bacterial flora mainly remained represented in equal numbers. We believe that these two methods should be employed in the bacteriological examination of the sinus: if the puncture has been positive the punctate alone should be analyzed, if negative lavage with sterile bouillon should be carried out. The lavage with bouillon must always be followed by lavage with saline in order to remove from the sinus an ideal medium for bacteria.

### CONCLUSION

On basis of our investigations, we may conclude that swabs from the ear in acute otitis media have a reduced diagnostic value if suppuration is not fairly abundant. Bacterial flora sensitive to penicillin predominates. In chronic inflammation the flora is mixed and broad spectrum antibiotics produce the best effect. In various pathological conditions of the tracheobronchial tree the flora varied greatly—hence only a combination of several antibiotics can produce certain results. Only the punctate or lavage with bouillon should be used in bacteriological examinations of sinus infection, for if lavage with saline is used the picture of infection with *H. influenzae* can easily be dissimulated. Concurrent bacteriological examinations of the nasal cavity and the maxillary sinuses have not yielded equal bacteriological findings—which speaks against the opinion that the bacteriological findings in the nasal mucosa can be a bacteriological indicator of the type of infection in the paranasal cavities.

### ZUSAMMENFASSUNG

Insgesamt wurden 516 Fälle von Erkrankungen des Mittelohrs, der Nase, Nebenhöhlen und spezifische und unspezifische Lungenerkrankungen bakteriologisch untersucht. Auf Grund unserer Untersuchungen können wir feststellen, dass der Abstrich aus dem Ohr bei akuter Mittelohrentzündung einen geringeren diagnostischen Wert hat, wenn die Sekretion nicht starker ausgeprägt ist. Es überwiegt die auf Penicillin reagierende bakterielle Flora. Bei chronischer Entzündung ist diese Flora gemischt und die beste Wirkung erzielen Antibiotika mit weitem Spektrum. Die bakterielle Flora ist bei verschiedenen pathologischen

Zuständen des tracheobronchialen Baumes sehr verschieden und nur eine Kombination mehrerer Antibiotika kann erfolgreich wirken. Bei bakteriologischen Untersuchungen der Nasenhöhleninfektion müssen wir entweder das reine Penicillin oder eine Waschung mit Bouillon gebrauchen, weil uns sonst bei Waschungen mit Kochsalzlösung oft das richtige Bild der bakteriologischen Infektion mit *Haemophilus influenzae* verborgen bleibt. Die parallelen bakteriologischen Untersuchungen der Nase und der Maxillarrhöhlen ergaben nicht die gleichen bakteriologischen Befunde, was gegen die Meinung spricht, dass der bakteriologische Befund der Nasenschleimhaut ein bakteriologischer Indikator der Paranasalinfektion sein kann.

## REFERENCES

- KONTZAKOVA, A. E., 1939. Investigations of the bacterial flora of the respiratory tract. *Acta Otolaryng.* (Stockh.) Suppl. 159.  
 MROSOVIĆ, B., BABIĆ, L., and BUKIĆ, V., 1965. Bacteriological investigations I. bronchoscopy. *Laryngoscope* 75, 66.

Z. KRAJINA, M.D.

*Of Rhinolaryngological Clinic  
 University of Zagreb, Zagreb  
 Yugoslavia*

Received April 20 1968

## TEMPORARY ARTERIAL OBSTRUCTION

### *Effect on Perilymph Oxygen and Microphonics*

M TSUNOO and H B PERLMAN

*From the Department of Otolaryngology University of Chicago Chicago Ill.  
U.S.A*

The principal source of oxygen to the cochlea is the arterial blood supply. When blood flow (oxygen delivery) is stopped the reserves of oxygen in the cochlea are very limited and begin to fall in a few seconds. The oxygen reserves drop in a linear fashion and are exhausted in about 100 seconds. The oxygen reserve in the cochlea can maintain normal cochlear function (microphonic output) for only a few seconds after blood flow stops. After this cochlear function drops reaching a 50% level in 40 seconds. With return of blood flow cochlear oxygen tension recovers in a linear manner while cochlear function recovers more rapidly. Full recovery of normal blood flow normal oxygen reserves and cochlear function can be obtained even after 8 minutes of obstruction of the internal auditory artery. When blood flow resumes the initial velocity may be two to three times normal. In a short period (i.e. 100 seconds) depending on the duration of the obstruction, blood flow velocity returns to normal. This is an indication of the capacity of this vascular bed for autoregulation.

Significant interrelations between blood flow, oxygen availability and function have been found in the brain, heart, kidney and skeletal muscle (Hirsch *et al.*, 1961; Chance *et al.*, 1962; Mayer *et al.*, 1962; Sayen *et al.*, 1958; Kramer & Deetjen, 1964; Stainsby & Otis, 1964; Lassen, 1964). Additional information about cell function has been obtained with locally perfused organs and in excised tissues in relation to oxygen utilization, substrates and enzymes involved, and function, i.e. brain slices and homogenates, nerves (Gerard, 1938), sympathetic ganglia (Larrabee & Bronk, 1952), heart muscle cell suspensions, etc. Finally knowledge of *in situ* rapid intracellular changes in respiration (cytochromes in mitochondria) associated with anoxia, apnoea and EEG changes have been obtained by use of microspectrophotometric methods (Chance *et al.*, 1962). The role of oxygen in cell function is thus becoming more clear.

Some experimentally produced changes in cochlear blood flow and oxygen delivery have been related to changes in cochlear function and to changes

This work was supported by Public Health Service grant No. NS00269-1 from the National Institutes of Health.

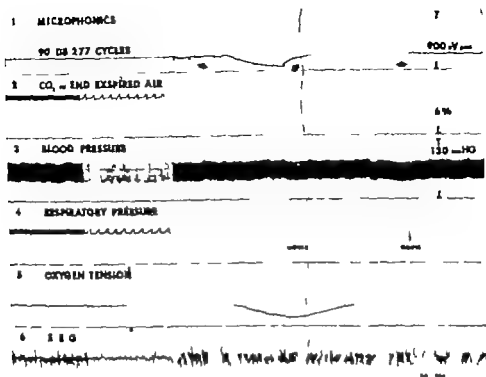


FIG. 1. Record of experiment in which cochlear blood flow was stopped for 90 sec.

in cochlear (perilymph) oxygen tension. These resulted from obstruction to the inferior cochlear vein, anoxia, asphyxia, hypercarbia, hypertension etc. (Taunoo & Perlman, 1965). The effect of sudden complete temporary interruption of all cochlear blood flow locally can also be examined because exposure and compression of the internal auditory artery is possible. The method has been described earlier (Kimura & Perlman, 1958). In this way acute transient local ischaemia of the cochlea can be produced without altering the general physiological condition of the animal.

For this study on the cochlea the effect of short periods of arterial occlusion (1-6 minutes) were studied since earlier experiments indicated that rapid severe reversible changes in function result when cochlear blood flow is completely interrupted for this time. Local and systemic changes are recorded simultaneously (see earlier reports).

### Findings

#### Blood flow

With proper orientation of the probe tip over the anterior inferior cerebellar artery immediate occlusion is obtained with a very small downward displacement of the micromanipulator (even a vessel of 500  $\mu$  diameter

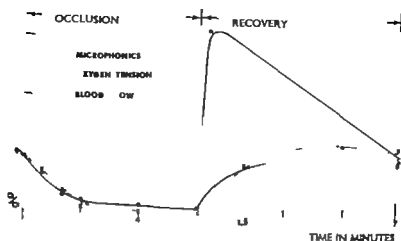


FIG. 2. Chart indicating simultaneous changes in microphonic output and blood flow rate during 6 minutes arterial occlusion and after return of blood flow expressed in per cent of normal preocclusion values.

with a blood pressure of 100 mm Hg can be occluded with 0.3 g force). This occlusion is indicated by the abrupt cessation of blood flow in the exposed strial vessels. Similarly with the micromanipulator a small elevation of the probe tip restores the blood flow at once. Occasionally flow did not return due to damage of the vessel at the site of compression.

Of interest is the prompt onset of supernormal flow on release of obstruction. The degree and duration of this period of supernormal flow was related to the duration of obstruction, i.e. return to normal flow in 7 minutes after 6 minutes of arterial obstruction. Immediately after release, flow rates may be two to three times normal. The absence of hemorrhage indicated gross integrity of the vessel walls with return of flow. The stagnant red cells, plasma, white cells and platelets were washed rapidly out of the strial capillaries, radiating arterioles, arteriovenous arcades and venules. Some dilation of the vessels was seen during the period of supernormal flow. All vessels remained filled during the brief periods of circulatory arrest.

### *Cochlear function*

Microphonic response from differential electrodes in the 3rd turn of the cochlea to a continuous non overloading acoustic stimulus (277 cycles 90 dB). With cochlear blood flow stopped, a normal microphonic response could not be sustained for longer than about 5 seconds. Then the response began to drop rapidly at first so that within 40 seconds it was reduced to 50% of the initial value. The response decline then became slower but was almost complete in 120 sec. Some further small drop could be seen however throughout a 6 minute obstruction. On return of blood flow—50% of the initial RMS value for microphonic output was rapidly restored in about 80 seconds. Both the initial rapid rise in microphonic and the slower later rise appeared to be uninfluenced by the length of obstruction up to 6 min.

TABLE 1 Recovery of microphonics oxygen tension and blood flow after arterial occlusion

Microphonics is expressed in m.v. mm. and oxygen tension and blood flow in percent of base line.

		of base line.				
Time, condition	Base line	Recovery min				
		0	1.5	3.0	5.0	7.0
<i>1.5 min occl.</i>						
Microphonics	340	100-200	300	340	340	
Oxygen tension	100	35	93	95	107	
Blood flow	100	280	270	124	102	
<i>2.6 min occl.</i>						
Microphonics	340	80-180	320	320	330	
Oxygen tension	100	15	85	115	125	
Blood flow	100	260	212	200	121	
<i>4.6 min occl.</i>						
Microphonics	340	40-120	260	320	320	300
Oxygen tension	100	10	190	120	130	120
Blood flow	100	310	272	280	171	97

Illustrated in Fig. 2.

utes. The recovery of microphonic response with return of blood flow was thus more rapid than its decline when the blood flow stopped. In the recovery period, unlike blood flow rates, no supernormal values were observed in the microphonic response. Indeed, occasionally full recovery of the response after obstruction did not occur during the period of observation (30 minutes).

Oxygen tension (in the perilymph of the scala vestibuli of the basal turn measured with a bare tipped 70  $\mu$  polarographic platinum electrode)

Oxygen tension began to drop almost immediately after blood flow stopped and continued to decline in a rather linear fashion for about 120 seconds when a plateau was reached corresponding to zero oxygen tension as subsequently determined with nitrogen breathing. The values were recorded a percent change, taking the level with air breathing and normal blood flow as 100%.

A 50% decline in oxygen tension was reached in about 40 to 50 seconds. On return of blood flow the oxygen tension rose in a similar manner to its drop, reaching the 50% level in about 40 seconds. This recovery rate appeared to be independent of the duration of the obstruction up to 6 minutes. The 100% level was reached in about 100 seconds after return of blood flow. A small degree of supernormal oxygen tension (13%) occasionally followed this and continued for 100 to 200 seconds depending on the duration of the obstruction.

## DISCUSSION

*Cochlear blood flow*

The behavior of the strial vessels and their contents during arrest and return of cochlear blood flow has been described in earlier experiments (Perlman *et al* 1959)

The larger cochlear arteries probably dilate in response to occlusion of the feeding vessel (internal auditory artery) and this results in a brief period of supernormal flow in the strial vessels on release of the probe. The reaction is a manifestation of autoregulation and has been recorded in many vascular beds, i.e. brain, kidney, heart. Other evidence for autoregulation of cochlear blood flow has been obtained (Perlman & Yamada 1967). The mechanisms for autoregulation of blood flow are complicated and continue to be investigated.

*Cochlear function*

The oxygen remaining in the cochlea after sudden arterial occlusion could sustain cochlear function (normal microphonic output) for only a few seconds. The output dropped (rapidly at first and then more slowly) in a manner as has been reported earlier (Perlman *et al* 1959, Konishi *et al* 1961). The dependence of cell function on oxygen supply has been widely studied. The recent investigations by Chance *et al* (1962) and his collaborators has thrown much light on the state of respiratory enzymes in the cell and oxygen utilization.

The recovery time of the microphonic response was more rapid than the survival time. Different enzyme systems controlling the decomposition and regeneration of metabolic reserves may be responsible for this.

*Cochlear oxygen tension*

The manner of calibration of the oxygen electrode in the cochlea has been previously described. No rhythmic fluctuations in perilymph oxygen tension or in blood flow was noted before, during, or after arterial occlusion. Davies & Bronk (1957), Clark *et al* (1958) and Silver (1965) report spontaneous fluctuations in oxygen tension with electrodes on the cortex. These were taken to indicate rhythmic changes in cortical blood flow.

With cessation of flow, zero oxygen tension levels were reached in about 120 seconds as all the oxygen of the cochlea was used up by the metabolizing cells. Much of the oxygen in the cell is used by the cytochrome system in mitochondria for respiration.

The rate of exhaustion of oxygen in the perilymph after occlusion of the internal auditory artery appears to be slower to reach zero (about 120 sec) than in the heart muscle on occlusion of a branch of the coronary artery (30 seconds) (Sayen *et al* 1958). Davies & Bronk (1958) report that local occlusion of an artery in the cortex resulted in a severe drop in oxygen to a sustained low value (5 mm Hg) in one second.



After  $pO_2$  values in the cochlea reach a zero level and complete arrest of blood flow continues no improvement can be obtained with the animal breathing 100% oxygen. Similarly if the internal auditory artery is permanently damaged as indicated by failure of blood flow to return on release of the probe, the  $pO_2$  values are not changed by respiring the animal with 100%  $O_2$ . The recovery of  $pO_2$  values in the perilymph and short period of supernormality with return of blood flow resembles that seen in arterial occlusions of the brain and heart. The recovery of oxygen tension on return of blood flow was more rapid in the heart (5 to 10 sec) and brain (12 sec) than in the cochlea (perilymph) 50-90 seconds.

### ZUSAMMENFASSUNG

Die hauptsächlichste Quelle des Sauerstoffes der Cochlea ist der arterielle Blutstrom. Wenn der Blutstrom (die Sauerstoff-Zugabe) ert gehalten wird, ist die Reserve so gering, dass er in wenigen Sekunden abfällt. Die Sauerstoff-Reserve fällt in unenormiger Weise ab und ist in ungefähr 100 Sekunden verbraucht. Diese Sauerstoff-Reserve in der Cochlea erhält die normalen microphonisch Potentiale der Cochlea nur für einige Sekunden nach der arteriellen Okklusion. Dann fällt die Funktion der Cochlea 50% in 10 Sekunden.

Nach dem Zurückkehren des Blutstromes stellt sich die Sauerstoff-Spannung der Cochlea wieder in einer unenormigen Weise ein, wegen der Funktion der Cochlea ist schnell wiederhergestellt ist. Sogar nach 6 Minuten Okklusion der Internal Auditory Arterie kann normaler Blutstrom, normale Sauerstoff-Reserve und normale Funktion der Cochlea wieder fortsetzen. Wenn der Blutstrom wieder anläßt, kann die anfängliche Verlustzeit zwei oder drei mal normal sein.

In einer kurzen Zeit (d. h., sek.) welches von der Länge der Okklusion abhängt, kehrt die normale Blutstrom-Verlustzeit zurück. Das deutet auf die autoregulation Fähigkeit dieser Gefäße.

### REFERENCES

- CLANCE, B. 1957 Cellular oxygen requirements. *Fed. Proc.* 16, 671.  
 CLANCE, B., COHEN, P., JOHNS, F. and SCHROEDER, B. 1962: Intracellular oxygen state in vivo. *Science* 137, 199.  
 CLARK, L. C., MICHAM, G. and FOX, R. 1958: Chronically implanted polarographic electrodes. *J. Appl. Physiol.* 23, 86.  
 DALLA, P. W. and BAO, K. D. W. 1957 Oxygen tension in mammalian brain. *Fed. Proc.* 16, 649.  
 GUNDEL, R. W. 1938: Anoxia and neural metabolism. *Arch. Neurol. Psychiat.* 40, 944.  
 HENNING, H., GLASCHMANN, A., KUNZE, H. and MANNING, V. 1961: Über die Beziehung zwischen  $O_2$ -Ausschüttung des Gehirns und  $O_2$ -Druck im Blut des Gehirns bei eingeschränkter und eingeschränkter Durchblutung. *Pflüger Arch. Ges. Physiol.* 273, 213.  
 KIRBY, R. and PERLMAN, H. B. 1958: Arterial obstruction of the labyrinth. *Ann. Otol.* 67, 5.  
 KORNHUBER, H. H., DILLER, R. A. and PERLMAN, H. B. 1961: Effect of anoxia on cochlear potentials. *J. Acoust. Soc. Amer.* 33, 349.  
 KRAUSE, K. and DEUTSCH, P. 1964: Oxygen consumption and redox in respiration in the mammalian kidney. *Oxygen in the animal organism*, p. 41. Pergamon Press, New York.

- LARRABEL, M G and BRONK, D W 1952: Metabolic requirements of sympathetic nerves. *Sympos Quant Biol* (Cold Spring Harbor) 17 245
- LASKIN, V A 1964: Autoregulation of cerebral blood flow *Autoregulation of blood flow* vol 1 p 201 American Heart Association Inc.
- MEYER, J S and GOTOH, T, TAKAKI, Y, HAMAGUCHI, H., ISHIKAWA, S., NOCHIMAT, F and SYMON, L., 1962 Regional cerebral blood flow and metabolism *in vivo* *Arch. Neurol.* 7 560
- PERLMAN, H B, KIMURA, R and FERNÁNDEZ, C., 1959 Experiments on temporary obstruction of the internal auditory artery *Laryngoscope* 69 591
- PERLMAN, H B and YAMADA, S 1967: Autoregulation of arterial blood flow 3rd Symposium on the Role of the Vestibular Organs in Space Exploration NASA, Feb. 1967
- SAVEN, J J, SHELDON, W F, PIERCE, G and KLOPFER, T 1968 Polarographic O<sub>2</sub> in the epicardial electrocardiogram and muscle contraction in perimital acute regional ischemia of the left ventricle. *Circulat Res* 22 779
- SILVER, I A 1965 Some observations on the cerebral cortex with an ultramicro-membrane-covered oxygen electrode. *Med Electron Biol Engng* 3 377
- STAINSBY, W N and OTIS, A B 1964 Blood flow blood oxygen tension, oxygen uptake and oxygen transport in skeletal muscle *Amer J Physiol* 206 855-864
- TSUNOO, M and PERLMAN, H B 1965 Cochlear oxygen tension Relation to blood flow and function *Acta Otolaryng* (Stockh.) 59 437

Dept of Otolaryngology  
University of Chicago  
Chicago Ill U.S.A

Received March 22 1968

## EOSINOPHILIC PAPILLARY CYSTADENOMA OF THE LARYNX

C. EKEDAH and L. B. SCHÜTTER

*From the Departments of Otolaryngology and Pathology Region Jukhuset Örebro Sweden*

Only a small number of reports on Eosinophilic Papillary Cystadenoma of the larynx has been made. The article describes two more cases. Treatment of the tumour is discussed.

Medical literature contains, as far as can be ascertained, reports on only 11 cases of eosinophilic papillary cystadenoma of the larynx. These cases have recently been presented by Steglich-Petersen (1966) who points out the difficulty in view of the small number of cases known, of making any definite conclusion regarding prognosis and treatment. In view of this it seems to be justified to present two more cases, one of which has been followed up for more than 3 years.

### Case 1

A 66-year-old male who consulted us in 1963 on account of increasing hoarseness during 2 years. The examination revealed an oedematous, pedicled, reddish polyp pendulating between the vocal cords. It was attached at the anterior commissure above the left vocal cord, and could be extirpated endoscopically. The histological diagnosis (elsewhere) was a benign polyp of the larynx, but it was also remarked that oncoocytes were observed here and there. A follow up examination about 2 years later showed a local recurrence but, in addition, both the aryepiglottic folds were dotted with disjunctive growths, one of which was removed for histological examination. Six months later a bean-sized tumour was extirpated at the anterior commissure under the right vocal cord. No progress has been observed at further check up during the last year however there is still a diffuse spread of minute growths over the aryepiglottic folds, the arytenoid region and the laryngeal surface of the epiglottis. On two further occasions, biopsy has been made from these.

In all seven biopsies during the period 1963-1966, microscopic examination revealed a typical papillary cystadenoma which, like the cases described in earlier published reports, was found to be made up of single-layer or (two-layer eosinophilic (picrinophilic in a haematoxylin van Gieson-stained section) predominantly large-celled epithelium, with more or less pronounced granulation of the cytoplasm. The epithelium formed both cysts and



FIG. 1 Papillary formation just outside the main cystic tumor Case 1 Haematoxylin-van Gieson 160

densely clustered papillary formations which here and there occurred also outside the cysts. The epithelium showed no atypia or other indications of malignancy

### Case 2

A 59 year-old male who consulted us in 1961 for hoarseness during 6 months. Examination of the larynx revealed a pea sized polyp located subglottally in the anterior commissure. A biopsy from this rather hard polyp gave only fragmentary material and at the microscopic examination (elsewhere) the growth was interpreted as a benign laryngeal polyp. Three months later further polypoid material was removed, but this was not examined histologically. In 1963, a recurrence, the size of a grain of rice was found in the anterior commissure. This was extirpated but the specimen was not examined histologically. In February 1967 i.e. 6 years after the first symptoms, the patient consulted us again on account of increasing hoarseness and upon examination a pea sized, broad based polyp was found subglottically at the anterior commissure. This was removed and was found to be a typical papillary oncocytic cystadenoma of the same type as in case 1.

### DISCUSSION

In case 2, it must be stated that only the extirpation in February 1967 can be regarded as radical and also that since there is no histological proof

that the same tumour was present at the different occasions of examination, it is still not possible to judge with any certainty whether or not there is a tendency for recurrences in this case. In case 1 on the other hand, the histological examination clearly revealed that the same type of tumour is present all the time. The local spread which has occurred is of particular interest. In the report from the first operation it is stated that "the stalk of the polyp is very hard and difficult to remove in its entirety". Among 31 cases of cystadenoma of the larynx described in medical literature there are seven in which the tumour contains oncocytes, large eosinophilic epithelial cells with granules in the cytoplasm. In no less than four of these cases has a local recurrence occurred, a circumstance which has not been found in those cystadenomas which lack oncocytes. In our first case, which belongs to the former morphological group, the tumour actually recurred, even in multiple form. The papillary glandular proliferation on the outside of the cyst in the case described above distinguishes the tumour from an ordinary retention cyst. We have gained the impression that tumours containing cells like those described above behave similarly i.e. have a marked tendency to recur especially in cases with pronounced extracystic, papillary glandular proliferation. In these patients, radical removal of the adenoma should be attempted in the first instance. If a local relapse occurs despite this, the therapy should be conservative, with check ups at frequent intervals, as long as the respiratory passage is not interfered with. Radical surgery resulting in laryngeal invalidity cannot be considered justifiable. Malignancy has not occurred in any of the cases described hitherto. In some cases, there has instead been some tendency to stagnation of the tumour growth.

# ZUSAMMENFASSUNG

Nur wenig Arbeiten über Eosinophile papilläre Cystadenomas im Kehlkopf sind geschrieben worden. Diese Arbeit beschreibt noch zwei Fälle. Die Behandlung wird diskutiert.

# REFERENCES

- CARP, O. A., 1903. Eosinophilic adenoma (oncocytoma) of the larynx. *Arch. Otolaryng. (Chic.)* 57 42.
- FELT, F. and ROWLAND, W. D. 1944. Cystadenoma of the larynx. *Arch. Otolaryng. (Chic.)* 49 443.
- HEATH, H. 1901. Eosinophilic granular-cell adenoma of the larynx. *J. Laryng.* 75 678.
- HENNING, W. and CHAMBERLAIN, A. J. 1934. Cystic adenomata of the parotid glands of Morgagni. *J. Laryng.* 49 324.
- KRUMHOLTZ, A. J. 1901. Cystadenoma of the parotid gland and larynx. *Arch. Otolaryng. (Chic.)* 73, 404.
- MOORE, L. and EDWARDS, C. M., 1929. Adenomata (glandular tumours) of the larynx. *J. Laryng.* 55, 85.
- MULLER, J. and ORNSTEIN, L. 1903. Laryngeal oncocytoma. *Act. Otolaryng. (Stockh.)* 69 239.

- NEW G and ENICH, J 1938: Benign tumours of the larynx. *Arch. Otolaryng* (Chic.) 22, 841
- NOTHMAN, H 1916 A case of laryngeal cyst composed of oncocytes and the appearance of oncocytes in the mucous membrane of the nose and larynx. *Acta Path. Microbiol. Scand* 23 473
- PICKERTON, P H and SWANSON BACK, J., 1961 Eosinophilic granular-cell ( oncocytic) cysts of the larynx *J Path Bact* 81 532.
- RAUER, B and THACKRAY A. C., 1953 Papillary cystadenoma of the larynx. *J Laryng* 67 609
- SOM M L., and PRIMER, R 1949 Oncocytic cystadenoma of the larynx. *Ann Otol. M.* 234
- STOLICH PETERSEN J 1966 Eosinophilic papillary cystadenoma of the larynx. *Acta Otolaryng* (Stockh) 62 505
- STEWART E. F 1960: Cystadenoma of larynx. *J Laryng* 74 325
- VOSTERN, K H 1958 Das onkocytäre Adenom des Kehlkopfes. *Arch Ohr Nas. Kehl pf* heilk 6 313

C. Ekedahl M.D

Dept of Otolaryngology

Regionalsjukhuset i Umeå Sweden

Received July 18 1968

## MEATOTYMPANOPLASTY WITH SPECIAL REFERENCE TO THE POSTOPERATIVE HEARING

T. NAITO, R. NAKAJIMA and T. NAKAMURA

*From the Department of Otorhinolaryngology, Osaka University Medical School  
Osaka, Japan*

We introduce our experience in "meatotympanoplasty" a tympanoplastic operation with the preservation of the ear canal. The outline of the procedure (elevation of the skin of the ear canal from the bony wall, a pedicle tube, retrograde removal of the bridge portion as needed, excystation of the mastoid pathology by retroauricular approach, reestablishment of middle ear conductive system, covering the ossicles with the connective tissue flap and repositioning the skin of ear canal over the flap. Postoperatively drying of the ear canal is attained early and the after care is unnecessary. It is also emphasized that the preservation of the normal ear canal is the method of choice from the standpoint of hearing gain. A favorable effect of the preservation of the normal ear canal upon the postoperative hearing was substantiated by clinical and experimental studies. Clinically the hearing was compared before and after packing the posterior fossa of the tympanoplastically operated ear canal. Experimentally the effect of the posterior fossa upon the resonance of ear canal was studied using a plaster ear model and dissected human temporal bones. These experiments confirmed that the normally constructed ear canal improved the hearing in higher frequencies by about 20 dB in comparison with that in the presence of posterior fossa.

It has been more than 15 years since tympanoplastic operations were first introduced by Wullstein (1952), Zöllner (1951), Goto (1953), and others. In a span of 15 years, a tremendous amount of fundamental and clinical work has contributed to the steady advancement of the art. We believe that the Japanese contribution in this field has been as valuable as those of European and American countries because we have a comparatively larger number of cases of chronic otitis media.

Considering the anatomical variability of the middle ear and the diversity of local findings, it is easily understood that no stereotyped technique of tympanoplasty can be applied to every individual case. Difficulty in tympanoplasty arises because one is always faced with two problems at the same time, namely excystation of pathology and improvement of hearing, the aims of which are often incompatible.

The authors have reported in the past several years on tympanoplastic operations which are more or less original as to the techniques of healing

plastik columella interposition myringoplasty and preservation of the bony ear canal

Among them what we call meatotympanoplasty a tympanoplasty with the preservation of the bony ear canal is our favorite technique and is the subject of this paper. It has been proved to be an excellent technique in view of postoperative hearing gain and easy or almost unnecessary after care when combined with the usage of connective tissue as an ear drum substitute.

### *Meatotympanoplasty*

We have practised this operation since the end of 1960. The primary concern of this operation is a morphological integration of the external ear canal. This is achieved by elevating the skin of the ear canal as a pedicle tube exenterating the middle ear mastoid cell pathologies, reconstructing the sound conductive system and then repositioning the skin on the preserved bony ear canal. Therefore, our meatotympanoplasty can be called a "meatus preserving operation".

A method intending to preserve the bony ear canal is by no means new. Bárány applied it to the radical operation in 1923. Bárány left the skin of the ear canal in place while the posterior wall of the bony ear canal being taken down. It can be said that there are two reasons which have stood in the way of further development of Bárány's method. The first is that before the advent of antibiotics, the method could not radically control the inflammation. The second is that the large mastoid cavity made by the radical operation was less secretory than that made by the present tympanoplastic operation. The reason for the second must be that the mastoid cavity of radically operated ear is lined with the extended skin of the ear canal, while the cavity made by tympanoplastic operation is covered with a heterotopical skin flap from the retroauricular region anterior thigh or upper arm, etc., and that the heterotopical skin is naturally different from the skin proper in glandular secretion, desquamation, resistance to bacteria and other stimulations.

It is quite reasonable that the preservation of a "fossaless" ear canal in tympanoplasty has received world wide acceptance, when we consider how troublesome it is to keep a large mastoid cavity clean and dry against retarded epithelialization, accumulation of desquamated mass, and repeated secretory dermatitidis. Wullstein and House also insist upon the preservation of the ear canal. A number of different measures against the posterior fossa are not mentioned here. One of such measures is the preservation of the posterior wall of the external ear canal (Vallo, 1961; Ouchi, 1960; Aini, 1963; Muta, 1964; Austin, 1961; McLaurin, 1961; Brunner, 1959; Vser, 1960; Jansen, 1963; Schnee, 1963; Farrlor, 1962; Ruyra, 1962; Palva, 1963 and others).

We would like to outline the operative procedures of meatotympanoplasty (Fig. 1).



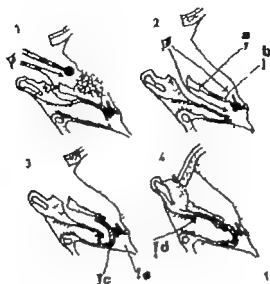


FIG. 1. Meatotympanoplasty of ear recess practice (horizontal section)

- 1 Retroauricular incision along the root of the pinna.
- 2 Elevation of the skin of the ear canal down to the ear drum as a pedicle tube.
- 3 Total exenteration of pathological mastoid cells with the posterior wall of the ear canal preserved (a1).
- 4 Management of the ossicular chain and oval window after the retrograde removal of the bridge portion, when necessary (b1).
- 5 Biting off the postero-inferior portion of the ear canal for better visualization and treatment of the hypotympanum and round window.
- 6 Management of the tubal orifice and its surroundings.
- 7 Filling up the middle ear cavity with pieces of gelatin sponge moistened with antibiotic solution (c1).
- 8 Making new ear drum of a small, 1 mm thick piece of connective tissue from the fascia temporalis or its neighbourhood (c1).
- 9 "Repositioning" of the skin sheath, its deep portion extending over the connective tissue flap (d1).
- 10 Packing the external ear canal with pieces of gelatin sponge.
- 11 Primary closure of retroauricular wound.

When hemostasis, and exenteration of the pathology are complete, tamponade of the closed mastoid cavity is not necessary. Tubal air passage is begun from the 4th to 5th postoperative day with minimal pressure. About 2 weeks postoperatively the packing in the ear canal is removed, and thereafter it is kept without packing. Epithelialization down to the newly implanted ear drum becomes completed and the ear canal dries up in 2 to 3 weeks.

the columella interposition myringoplasty and preservation of the ear canal among them what we call meatotympanoplasty a tympanoplasty with preservation of the bony ear canal is our favorite technique and is effective for hearing. It has been proved to be an excellent technique for postoperative hearing gain and easy or almost unnecessary maintenance combined with the usage of connective tissue as an ear drum.

### *Meatotympanoplasty*

We have practiced this operation since the end of 1960. The present operation is a morphological integration of the external ear canal achieved by elevating the skin of the ear canal as a periauricular flap, separating the middle ear mastoid cell pathologies, reconstructing the conductive system and then repositioning the skin on the posterior wall of the bony ear canal. Therefore our meatotympanoplasty can be called a "conservative preserving operation."

A method intending to preserve the bony ear canal is by no means new. Barany applied it to the radical operation in 1923. Barany left the skin of the ear canal in place while the posterior wall of the bony ear canal was taken down. It can be said that there are two reasons which have stood in the way of further development of Barany's method. The first is that before the advent of antibiotics, the method could not radically control the inflammation. The second is that the large mastoid cavity made by the radical operation was less secretory than that made by the present tympanoplasty operation. The reason for the second must be that the mastoid cavity of radically operated ear is lined with the extended skin of the ear canal while the cavity made by tympanoplasty operation is covered with a heterotopical skin flap from the retroauricular region, anterior thigh, or upper arm etc., and that the heterotopical skin is naturally different from the skin proper in glandular secretion, desquamation, resistance to bacteria, and other stimulations.

It is quite reasonable that the preservation of a fossaless ear canal in tympanoplasty has received world wide acceptance when we consider how troublesome it is to keep a large mastoid cavity clean and dry against retarded epithelialization, accumulation of desquamated mass, and repeated secretory dermatitis. Wullstein and House also insist upon the preservation of the ear canal. A number of different measures against the posterior fossa are not mentioned here. One of such measures is the preservation of the posterior wall of the external ear canal (Naito, 1961; Ouchi, 1960; Ando, 1963; Muta, 1964; Austin, 1961; McLaurin, 1961; Brunner, 1959; Silver, 1960; Jansen, 1963; Schnee, 1963; Farrior, 1962; Ruyra, 1962; Palva, 1963; and others).

We would like to outline the operative procedures of meatotympanoplasty (Fig. 1).

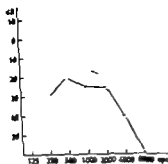


FIG. 3.

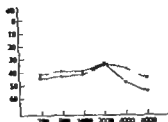


FIG. 4.

FIG. 3. Gradual improvement of postoperative hearing in fascia method. X—X preoperative hearing; X X, 3 weeks after operation X X 6 months after operation.

FIG. 4. A range of pre- and postoperative hearing in 30 cases of tympanoplasty X—X preoperative; X—X postoperative.

tympanoplasty can be also performed with free skin flap. At any rate, so many people, so many tastes" is also true as to the techniques of tympanoplasty.

### Postoperative Hearing Gain

1. *Statistical observations.* It seems quite controversial to evaluate each technique in terms of postoperative hearing gain, by putting many different cases together for the purpose of a statistical study, since the preoperative threshold, status of the ossicular chain, pathology in the middle ear and mastoid cells and many other factors are different from one case to another.

Supposedly, the effect of the posterior fossa of the ear canal upon postoperative hearing is of minor importance in comparison with re-establishment of the middle ear conductive system. Therefore, the effect of the

TABLE 1. General comparison of "sliding method" and "fascia method" of meatotympanoplasty

	Sliding method	Fascia method
Technique	Difficult	Easy
Skin graft	Occasionally needed	Unnecessary
Epithelialization	Slow	Fast
Postoperative perforation	Occasional	Seldom
Narrowing of the entrance of the ear canal	Occasional	—
Postoperative hearing gain	Early	Retarded
Postoperative care	Unnecessary	Unnecessary

This method is applicable to any of Wullstein's I to IV types on the condition that a certain special precaution should be exerted in placing the connective tissue flap for some cases of types I and IV. The newly implanted ear drum substitute gradually becomes thinner to be of normal thinness in 3 to 6 months.

### *Comparison of Fascia method with Sliding method"*

In the early practice of meatotympanoplasty we used an anteriorly allded pedicle skin flap to cover the middle ear cavity. The skin is carefully elevated from the posterior bony wall after incisions are made at the upper and lower corner of the posterior canal wall almost down to the annulus tympanicus (sliding fore method). Thus shifted skin serves favorably as an ear drum soon after the operation since the skin is acoustically thin enough from the beginning. On the other hand, the sliding fore method has following disadvantages:

(a) It takes time and is not necessarily easy to elevate the skin of the ear canal down to and over the annulus tympanicus without perforating it.

(b) The thin ear drum substitute tends to occasional perforation due to postoperative fluid retention in the middle ear.

(c) Skin graft is necessary to the area of skin defect in the posterior wall, where otherwise, natural epithelialization takes a fairly long time.

Fascia method brings about complete solution of those problems. However, the ear drum substitute as thick as 1 mm retards postoperative hearing gain particularly in higher frequencies. Hearing gain gradually manifests itself in 3 to 6 months.

Fig. 2 is the comparison of fascia method and sliding method in terms of hearing gain of 1 month after the meatotympanoplasty. In such early postoperative days, sliding method is superior to fascia method.

Fig. 3 is a demonstration of gradual improvement of hearing even 6 months after fascia method. Such is often the case and indicates the necessity of further follow up.

Table 1 is a general comparison of the two methods. One might as well choose either of them according to the individual case encountered. Our recent choice is fascia method in the majority of cases. Naturally meato-

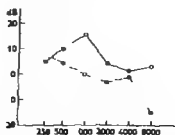


FIG. 2. Comparison of hearing gain by fascia method and sliding method (a range of 10 cases each). O—O sliding method, O---O fascia method.

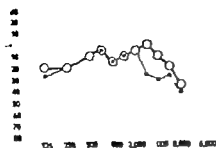


Fig. 7

Fig. 7 Change of hearing before and after packing of posterior fossa with chicle.  $\bullet$ — $\bullet$  Before packing;  $\circ$ — $\circ$  after packing.

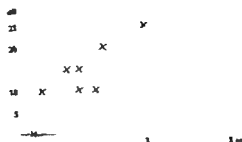


Fig. 8

Fig. 8. Relation between the volume of posterior fossa and hearing gain at 3000 cps when the fossa is filled.  $\times$  0 dB Pretreatment threshold.

**3 Experiments using plaster ear models and dissected human temporal bones** To make a quantitative analysis of the above-mentioned experiments, we performed the following observations using plaster ear models and dissected human temporal bones. Models of the pinna and ear canal were made of plaster. A receiver was placed against the auricle and the sound pressure was measured with the condenser microphone which was set at the point corresponding to ear drum. The same observation was made with the models, the posterior wall of which was carved in a manner of classical tympanoplasty. The volume of the fossa was one to four ml. The resulting hearing loss in higher frequencies was, as shown in Fig. 10 in proportion to the volume of the posterior fossa.

The same experiment done in a free field gave essentially the same

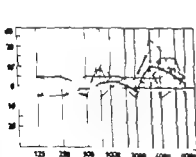


Fig. 9

Fig. 9 Change in hearing after the packing of the posterior fossa in 10 cases of tympanoplasty (thick line average of 10 cases) 0 dB Pretreatment threshold.

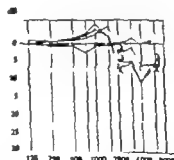


Fig. 10.

Fig. 10. Relation between the volume of the posterior fossa and hearing loss in ear model experiment. The numbers 1 to 4 on the lines correspond to the volume of the posterior fossa of 1 to 4 ml. 0 dB Sound pressure with the normal ear canal.



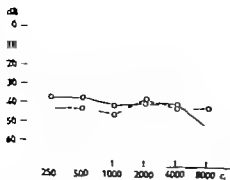


Fig. 5

Fig. 5. A range of pre and postoperative hearing of 20 cases of meatotympanoplasty. O---O Preoperative O—O postoperative

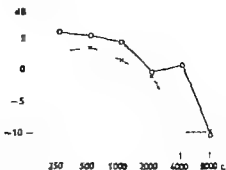


Fig. 6

Fig. 6. Comparison of hearing gain in two groups (20 cases each) 0 dB, No hearing + gain of hearing - loss of hearing x---x tympanoplasty O—O meatotympanoplasty

posterior fossa upon hearing cannot be duly evaluated when one merely compares the hearing of two groups, the group of meatotympanoplasty with normal ear canal and the group of classical tympanoplasty with a large posterior fossa

Admitting the above mentioned disadvantages, postoperative hearing of the two groups was statistically observed. Materials were 20 cases of tympanoplasty and the same number of meatotympanoplasty which had been all operated upon in our clinic in 1963 (Figs. 4, 5 and 6)

It was noted from the comparison that meatotympanoplasty is better as far as the improvement of hearing at 4000 cps is concerned. This result encouraged us to do the following experiments

2 *Clinical experiments* Mixture of various factors in the above-mentioned statistical observation makes it difficult to pick up the effect of the posterior fossa upon hearing in its pure form

As the next step therefore, we performed the following experiments. The large posterior fossa of the ear canal, which had been operated upon according to classical tympanoplasty was filled up with chicle (the material of chewing gum) to such an extent that the ear canal was almost rebuilt but that the vibration of the ear drum was not disturbed at all and then the hearing was compared before and after the treatment. The experiment, which in my opinion excluded many factors from the middle and inner ear might give an answer to the question.

Fig. 7 is an example. It shows a marked improvement of hearing in higher frequencies, 25 dB at 3000 cps and 20 dB at 4000 cps, while there was no shifting of threshold in lower frequencies.

The packing of the posterior fossa, however does not always favorably influence the hearing, but the general rule is that the larger the posterior fossa, the greater the hearing gain by its packing (Fig. 8)

Fig. 9 indicates the average hearing gain of 10 cases. 15 dB at 3000 cps and 10 dB at 4000 cps

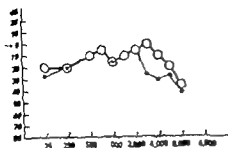


Fig. 7

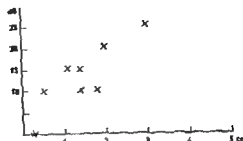


Fig. 8

Fig. 7 Change of hearing before and after packing of posterior fossa with bone.  
 ●—● Before packing ○—○ after packing

Fig. 8 Relation between the volume of posterior fossa and hearing gain at 3000 cps when the fossa is filled. p. 0 dB: Pretreatment threshold.

3. *Experiments using plaster ear models and dissected human temporal bones* To make a quantitative analysis of the above-mentioned experiments, we performed the following observations using plaster ear models and dissected human temporal bones. Models of the pinna and ear canal were made of plaster. A receiver was placed against the auricle and the sound pressure was measured with the condenser microphone which was set at the point corresponding to ear drum. The same observation was made with the models, the posterior wall of which was carved in a manner of classical tympanoplasty. The volume of the fossa was one to four ml. The resulting hearing loss in higher frequencies was, as shown in Fig. 10, in proportion to the volume of the posterior fossa.

The same experiment done in a free field gave essentially the same

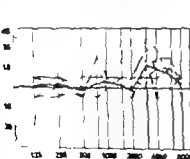


Fig. 9

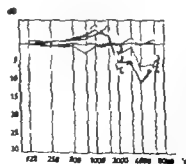
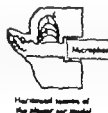


Fig. 10.

Fig. 9 Change of hearing after the packing of the posterior fossa in cases of tympanoplasty (thick line: average of 10 cases) p. 0 dB: Pretreatment threshold.

Fig. 10. Relation between the volume of the posterior fossa and hearing loss in ear model experiment. The numbers 1 to 4 on the lines correspond to the volume of the posterior fossa at 1 to 4 ml. 0 dB: Sound pressure with the normal ear canal.



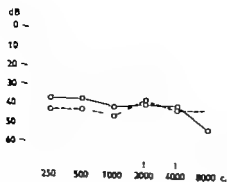


FIG. 5

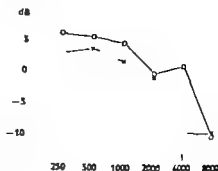


FIG. 6.

FIG. 5 Average of pre and postoperative hearing of 20 cases of meatotympanoplasty  
 O---O Preoperative O—O postoperative.

FIG. 6 Comparison of hearing gain in two groups (20 cases each) 0 dB, No change  
 + gain of hearing - loss of hearing x---x tympanoplasty O—O meatotympanoplasty

posterior fossa upon hearing cannot be duly evaluated when one merely compares the hearing of two groups, the group of meatotympanoplasty with normal ear canal and the group of classical tympanoplasty with a large posterior fossa.

Admitting the above-mentioned disadvantages, postoperative hearing of the two groups was statistically observed. Materials were 20 cases of tympanoplasty and the same number of meatotympanoplasty which had been all operated upon in our clinic in 1963 (Figs 4, 5 and 6).

It was noted from the comparison that meatotympanoplasty is better as far as the improvement of hearing at 4000 cps is concerned. This result encouraged us to do the following experiments.

**2 Clinical experiments** Mixture of various factors in the above-mentioned statistical observation makes it difficult to pick up the effect of the posterior fossa upon hearing in its pure form.

As the next step therefore, we performed the following experiments. The large posterior fossa of the ear canal, which had been operated upon according to classical tympanoplasty was filled up with chicle (the material of chewing gum) to such an extent that the ear canal was almost rebuilt but that the vibration of the ear drum was not disturbed at all, and then the hearing was compared before and after the treatment. The experiment, which, in my opinion, excluded many factors from the middle and inner ear might give an answer to the question.

Fig. 7 is an example. It shows a marked improvement of hearing in higher frequencies, 25 dB at 3000 cps and 20 dB at 4000 cps, while there was no shifting of threshold in lower frequencies.

The packing of the posterior fossa, however, does not always favorably influence the hearing, but the general rule is that the larger the posterior fossa, the greater the hearing gain by its packing (Fig. 8).

Fig. 9 indicates the average hearing gain of 10 cases: 15 dB at 3000 cps and 10 dB at 4000 cps.



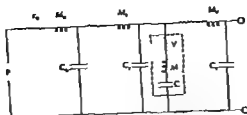


FIG. 13. Acoustic circuit of external ear canal.  $P$  Effective sound pressure;  $M$   $C$  acoustic resistance, inductance, and capacity at the entrance of external ear canal;  $M$   $C$  acoustic resistance, inductance, and capacity of ear canal;  $M$   $C$  acoustic resistance, inductance, and capacity of posterior fossa.

From an acoustic point of view the external ear canal is thought to be a tube and its acoustic circuit can be assumed to be as shown in Fig. 13.

The acoustic impedance caused by the air in the posterior fossa connects as a parallel circuit with the acoustic circuit of the external ear canal. (The acoustic impedance caused by the posterior fossa is encircled by a dotted line.)

It is reasonable to assume that this circuit system has a resonant frequency ( $f = 1/2\pi\sqrt{MC}$ ) and the sound near the resonant frequency is partly shunted, resulting in hearing loss. The volume of the posterior fossa made by classical tympanoplasty is maximum 3 ml, and the resonant frequency of the circuit happens to be in the range of 3000 to 4000 cps. That is how the conduction of the sound in that range is disturbed.

## CONCLUSION

It is needless to say that a careful treatment of the middle ear and mastoid is important, but the maintenance of a normal ear canal is equally important from the viewpoint of postoperative care and hearing. As far as the management of the mastoid cells is concerned, some people insist upon radical extirpation of pathology, the others prefer to treat as conservatively as possible and still others take an intermediate stand. In classical tympanoplasty desirable radical extirpation of mastoid pathology was necessarily accompanied by a large posterior fossa, which made postoperative care very troublesome.

More conservative management of the mastoid cells leaves the control of their inflammation largely to a natural healing process with minimal sacrifice of the ear canal. However, this conservativeness inevitably involves some risk of recurrence of inflammation. The same holds true as to the management of middle ear pathology: removal of pathological mucosa is occasionally followed by adhesion of the ear drum substitute to the promontory while preservation of the mucosa is not without the risk of recurrence of inflammation.

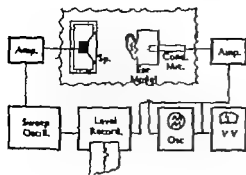


FIG. 11

FIG. 11 Equipment for the test in a free field.

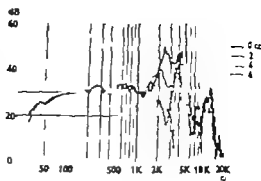


FIG. 12.

FIG. 12 Change of the sound pressure level with ear drum in correspondence to the volume change of the posterior fossa.

results as in the experiment using receiver that is, decrease of sound pressure in higher frequencies (Figs. 11 and 12)

Then the mastoid cavity of the dissected human temporal bone was opened into the ear canal by removing the posterior wall of the ear canal, and the change in sound pressure was measured on the outer surface of the ear drum (Figs. 13 and 14)

Just as in the preceding experiments, the posterior fossa elevated hearing threshold by 15 dB in 3000 to 4000 cps range, and the threshold elevation was proportional to the volume of the posterior fossa.

From the above-mentioned clinical and experimental observations, we are convinced that the posterior fossa definitely exerts an unfavorable effect upon hearing in 3000 to 4000 cps range

### Mechanism of Hearing Loss with Posterior Fossa

How the posterior fossa gives rise to hearing loss? A plausible explanation is that the sound is partly absorbed by the air in the posterior fossa on its way of transmission to the ear drum.

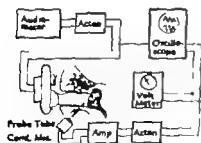


FIG. 13

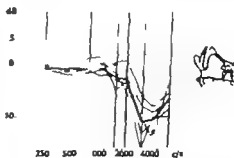


FIG. 14.

FIG. 13. Method of the experiment using a dissected human temporal bone

FIG. 14. Effect of the posterior fossa upon hearing in dissected human temporal bones (thick line: average of 11 cases; 0 dB: Sound pressure with normal ear canal)

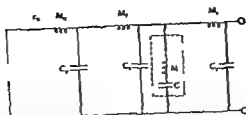


FIG. 15. Acoustic circuit of external canal.  $P$  Effective sound pressure;  $R_1, R_2, R_3$  acoustic resistance, inductance, and capacity at the entrance of external ear canal;  $M, C$  acoustic resistance, inductance, and capacity of ear canal;  $C_1, C_2$  acoustic resistance, inductance, and capacity of posterior fossa.

From an acoustic point of view the external ear canal is thought to be a tube, and its acoustic circuit can be assumed to be as shown in Fig. 15.

The acoustic impedance caused by the air in the posterior fossa connects as a parallel circuit with the acoustic circuit of the external ear canal (The acoustic impedance caused by the posterior fossa is encircled by a dotted line).

It is reasonable to assume that this circuit system has a resonant frequency ( $f = 1/2\pi\sqrt{MC}$ ) and the sound near the resonant frequency is partly shunted resulting in hearing loss. The volume of the posterior fossa made by classical tympanoplasty is maximum 5 ml and the resonant frequency of the circuit happens to be in the range of 3000 to 4000 cps. That is how the conduction of the sound in that range is disturbed.

## CONCLUSION

It is needless to say that a careful treatment of the middle ear and mastoid is important, but the maintenance of a normal ear canal is equally important from the viewpoint of postoperative care and hearing. As far as the management of the mastoid cells is concerned, some people insist upon radical extirpation of pathology, the others prefer to treat as conservatively as possible and still others take an intermediate stand. In classical tympanoplasty desirable radical extirpation of mastoid pathology was necessarily accompanied by a large posterior fossa, which made postoperative care very troublesome.

More conservative management of the mastoid cells leaves the control of their inflammation largely to a natural healing process with minimal sacrifice of the ear canal. However this conservativeness inevitably involves some risk of recurrence of inflammation. The same holds true as to the management of middle ear pathology: removal of pathological mucosa is occasionally followed by adhesion of the ear drum substitute to the promontory while preservation of the mucosa is not without the risk of recurrence of inflammation.

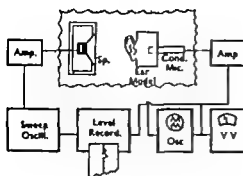


FIG. 11

FIG. 11 Equipment for the test in a free field

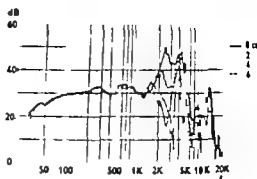


FIG. 12

FIG. 12 Change of the sound pressure level at the ear drum in correspondence to the volume change of the posterior fossa

results as in the experiment using receiver that is, decrease of sound pressure in higher frequencies (Figs. 11 and 12)

Then the mastoid cavity of the dissected human temporal bone was opened into the ear canal by removing the posterior wall of the ear canal, and the change in sound pressure was measured on the outer surface of the ear drum (Figs. 13 and 14)

Just as in the preceding experiments, the posterior fossa elevated hearing threshold by 15 dB in 3000 to 1000 cps range and the threshold elevation was proportional to the volume of the posterior fossa

From the above mentioned clinical and experimental observations, we are convinced that the posterior fossa definitely exerts an unfavorable effect upon hearing in 3000 to 4000 cps range

### Mechanism of Hearing Loss with Posterior Fossa

How the posterior fossa gives rise to hearing loss? A plausible explanation is that the sound is partly absorbed by the air in the posterior fossa on its way of transmission to the ear drum

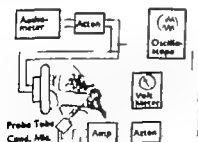


FIG. 13

FIG. 13. Method of performing the experiment

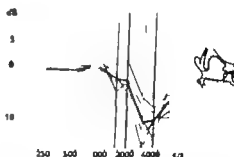


FIG. 14

FIG. 14 Effect of the posterior fossa upon the hearing of the dissected human temporal bone (thick line: average of five cases; 0 dB: Sound pressure with normal case)

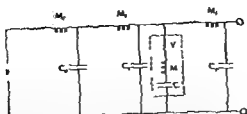


Fig. 15. Acoustic circuit of external ear canal.  $P$  Effective sound pressure;  $M_e$   $C_e$  acoustic resistance, inductance, and capacity at the entrance of external ear canal;  $M_c$   $C_c$  acoustic resistance, inductance, and capacity of ear canal;  $M_p$   $C_p$  acoustic resistance, inductance, and capacity of posterior fossa.

From an acoustic point of view the external ear canal is (thought to be a tube, and its acoustic circuit can be assumed to be as shown in Fig. 15.

The acoustic impedance caused by the air in the posterior fossa connects as a parallel circuit with the acoustic circuit of the external ear canal. (The acoustic impedance caused by the posterior fossa is encircled by a dotted line.)

It is reasonable to assume that this circuit system has a resonant frequency ( $f = 1/2\pi \sqrt{MC}$ ) and the sound near the resonant frequency is partly shunted, resulting in hearing loss. The volume of the posterior fossa made by classical tympanoplasty is maximum 5 ml, and the resonant frequency of the circuit happens to be in the range of 3000 to 4000 cps. That is how the conduction of the sound in that range is disturbed.

### CONCLUSION

It is needless to say that a careful treatment of the middle ear and mastoid is important, but the maintenance of a normal ear canal is equally important from the viewpoint of postoperative care and hearing. As far as the management of the mastoid cells is concerned, some people insist upon radical extirpation of pathology the others prefer to treat as conservatively as possible and still others take an intermediate stand. In classical tympanoplasty desirable radical extirpation of mastoid pathology was necessarily accompanied by a large posterior fossa, which made postoperative care very troublesome.

More conservative management of the mastoid cells leaves the control of their inflammation largely to a natural healing process with minimal sacrifice of the ear canal. However this conservativeness inevitably involves some risk of recurrence of inflammation. The same holds true as to the management of middle ear pathology removal of pathological mucosa is occasionally followed by adhesion of the ear drum substitute to the promontory while preservation of the mucosa is not without the risk of recurrence of inflammation.

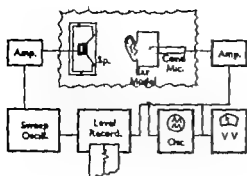


FIG. 11

FIG. 11 Equipment for the test in a free field.

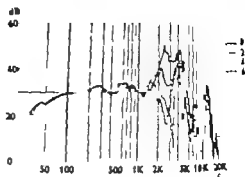


FIG. 12.

FIG. 12 Change of the sound pressure level at the ear drum in correspondence to the volume change of the posterior fossa

results as in the experiment using receiver that is, decrease of sound pressure in higher frequencies (Figs. 11 and 12)

Then the mastoid cavity of the dissected human temporal bone was opened into the ear canal by removing the posterior wall of the ear canal, and the change in sound pressure was measured on the outer surface of the ear drum (Figs. 13 and 14)

Just as in the preceding experiments, the posterior fossa elevated hearing threshold by 15 dB in 3000 to 4000 cps range and the threshold elevation was proportional to the volume of the posterior fossa

From the above-mentioned clinical and experimental observations, we are convinced that the posterior fossa definitely exerts an unfavorable effect upon hearing in 3000 to 4000 cps range.

### *Mechanism of Hearing Loss with Posterior Fossa*

How the posterior fossa gives rise to hearing loss? A plausible explanation is that the sound is partly absorbed by the air in the posterior fossa on its way of transmission to the ear drum

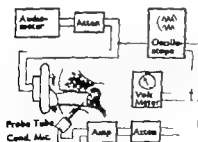


FIG. 13

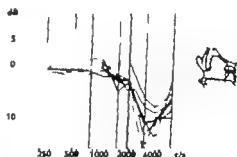


FIG. 14.

FIG. 13. Method of experiment using a dissected human temporal bone

FIG. 14. Effect of the posterior fossa upon hearing in dissected human temporal bone (thick line = average of five cases of 0 dB sound pressure with normal ear canal)

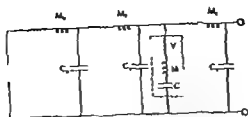


Fig. 16. Acoustic circuit of external ear canal.  $P$  Effective sound pressure;  $M_e, C_e$  acoustic resistance, inductance, and capacity at the entrance of external ear canal;  $M_c, C_c$  acoustic resistance, inductance, and capacity of ear canal;  $M_p, C_p$  acoustic resistance, inductance, and capacity of posterior fossa.

From an acoustic point of view the external ear canal is thought to be a tube, and its acoustic circuit can be assumed to be as shown in Fig. 15.

The acoustic impedance caused by the air in the posterior fossa connects as a parallel circuit with the acoustic circuit of the external ear canal. (The acoustic impedance caused by the posterior fossa is encircled by a dotted line.)

It is reasonable to assume that this circuit system has a resonant frequency ( $f = 1/2\pi\sqrt{M_c C_c}$ ) and the sound near the resonant frequency is partly shunted, resulting in hearing loss. The volume of the posterior fossa made by classical tympanoplasty is maximum 5 ml, and the resonant frequency of the circuit happens to be in the range of 3000 to 4000 cps. That is how the conduction of the sound in that range is disturbed.

### CONCLUSION

It is needless to say that a careful treatment of the middle ear and mastoid is important, but the maintenance of a normal ear canal is equally important from the viewpoint of postoperative care and hearing. As far as the management of the mastoid cells is concerned, some people insist upon radical extirpation of pathology the others prefer to treat as conservatively as possible and still others take an intermediate stand. In classical tympanoplasty desirable radical extirpation of mastoid pathology was necessarily accompanied by a large posterior fossa, which made postoperative care very troublesome.

More conservative management of the mastoid cells leaves the control of their inflammation largely to a natural healing process with minimal sacrifice of the ear canal. However this conservativeness inevitably involves some risk of recurrence of inflammation. The same holds true as to the management of middle ear pathology: removal of pathological mucosa is occasionally followed by adhesion of the ear drum substitute to the promontory while preservation of the mucosa is not without the risk of recurrence of inflammation.

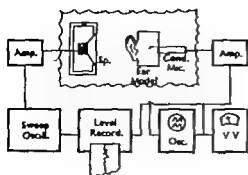


FIG. 11

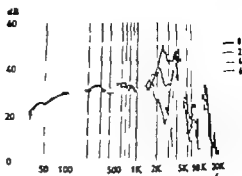


FIG. 12

FIG. 11 Equipment for the test in a free field.

FIG. 12 Change of the sound pressure level at the ear drum in correspondence to the volume change of the posterior fossa

results as in the experiment using receiver that is, decrease of sound pressure in higher frequencies (Figs. 11 and 12)

Then, the mastoid cavity of the dissected human temporal bone was opened into the ear canal by removing the posterior wall of the ear canal and the change in sound pressure was measured on the outer surface of the ear drum (Figs. 13 and 14)

Just as in the preceding experiments, the posterior fossa elevated hearing threshold by 15 dB in 3000 to 4000 cps range and the threshold elevation was proportional to the volume of the posterior fossa.

From the above-mentioned clinical and experimental observations, we are convinced that the posterior fossa definitely exerts an unfavorable effect upon hearing in 3000 to 4000 cps range

### Mechanism of Hearing Loss with Posterior Fossa

How the posterior fossa gives rise to hearing loss? A plausible explanation is that the sound is partly "absorbed" by the air in the posterior fossa on its way of transmission to the ear drum

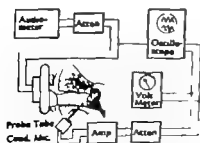


FIG. 13

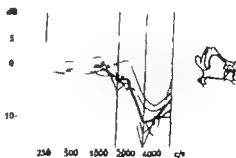


FIG. 14

FIG. 13. Method of experiment using a dissected human temporal bone

FIG. 14 Effect of the posterior fossa upon hearing in dissected human temporal bones (thick line average of five cases) 0 dB Sound pressure with normal ear canal



dung des Trommelfells mit Bindegewebe-Transplantation und Reponierung der äußeren Gehörgangshaut auf bindegewebige Transplantate. Nach der Operation ist die Operationswunde schnell trocken und die Nachbehandlung ist unnötig. Es muss noch betont werden, dass die Erhaltung des normalen äußeren Gehörganges nach der tympanoplastischen Operation der wichtigste Punkt in Bezug auf Hörverbesserung ist. Der gute Effekt durch die Erhaltung des normalen äußeren Gehörganges über das postoperative Gehör wurde mit klinischen und experimentellen Versuchen bemerkt. Kikuchi wurde das Gehör vor und nach Plombierung des "chicle" in der Operatihöhle der mastoidalen Zellen, d. h. ohne und mit dem relativ normalen äußeren Gehörgang geprüft. Experimentell wurde der Effekt der Operationshöhle über Resonanzphänomenen mit dem modellierten Ohr und menschlichen temporalen Knochen untersucht. Dieses Experiment hat gezeigt, dass normal gebildeter äußerer Gehörgang besseren Gehörgewinn, etwa 28 db, in hohen Frequenzen erzielt als der beim Zurückbleiben des Operationshöhle erfolgt.

## REFERENCES

- Arata, K., 1963 Improvement of mastoplastie method with particular reference to the after care. *O.J.L. Tokyo*, 8, 192.
- Altura, D. F. 1961 A new system of tympanoplasty using vein graft. *Laryngoscope* 71 496.
- Bischof, R., 1923 Die Radikaloperation des Ohrs oder Gehörgangsplastik Franz Deitcke, Leipzig und Vienna.
- Bor, W., M., 1936 Erhaltung und Restaurierung des Gehörganges bei Tympanoplastiken. *Wochschr. Ohrenheilk.* 83 1
- Farnson, J. B., 1963 Stapedectomy and tympanoplasty *Ill. Arch. Otolaryng* (Chic.) 6 335.
- Go, O., K., 1953 Tympanoplasty *Proct. Otol. Kyoto* 46 632.
- Goto, T. 1961 Treatment of the mastoid cells and difficulty of postoperative pathogenesis of the mastoid cavity in tympanoplasty *Jap. J. Otol. Tokyo* 31 1811
- 1962 The extent of mastoid cell removal *Otolaryng* (Tokyo) 31 341
- Hasekura, Y. 1962 General consideration of the methods of tympanoplasty and their trends. *Otolaryng* (Tokyo) 31 833.
- Hayashi, H., 1961 Trommelfellplastik mit Felleingewebe am Mastoideum Temporale nach Begründung der äußeren Gehörgangs- od. HVO Wegweise (Berl.) 8 126.
- 1962 Erfahrungen mit freier transplantiertem HVO-Bindegewebe des Mastoideum Temporale bei Tympanoplastik und Verkleinerung der Radikalhöhle Kiefergelenkbrüche vom Stapes zum inneren Trommelfell Brand. *Z. Laryng.-Rhinol. Otol* 31 161
- Horiuchi, H. 1948 Functional restoration of tympanoplasty *Arch. Otolaryng* (Chic.) 73 301
- Jensen, C., 1962 Cartilage-tympanoplasty *Laryngoscope* 72, 1233.
- Kikuchi, Y. 1934 Modified radical operation with the preservation of hearing. *Proct. Otol. Kyoto*, 47 80.
- Kikuchi, Y. 1960 Experimental study on pathophysiology of middle ear conductive deafness. *J. J. Otol. Tokyo* 33, 813
- Kikuchi, Y. T. 1962 Problem of the establishment of proper space beneath the eardrum of tympanoplasty *Otolaryng* (Tokyo) 31, 833.
- Matsuda, H., 1960 La tympanoplastie. *Rev. Proct* 10 2141.
- Mitsuda, Y., 1961 A technique of tympanoplasty Preservation of the bony canal with use of vein grafts. *Laryngoscope* 71 118.

We feel that meatotympanoplasty gives a solution to the above-mentioned contradictory problems: the pathological cells can be radically extirpated with no deformity of the ear canal and adhesive process of the middle ear cavity is avoided by packing the middle ear cavity thickly with pieces of gelatin sponge.

Meatotympanoplasty has of course its own problems to be solved. The first of them is the problem of a large dead space in the mastoid process. In general principle of surgery formation of a dead space is considered undesirable from the viewpoint of fluid retention and infection. But practically we have experienced no disadvantages such as rupture of retroauricular wound or secondary perforation of ear drum substitute. Presumably this might be due to drainage through the eustachian tube and osseous structure of dead space wall.

Further follow up is of course necessary to observe the destiny of a large mastoid cavity and its effect upon air conduction.

The second problem is the use of the fascia as an ear drum substitute. The fascia used should be fairly thick so as not to be perforated easily. The thickness retards the improvement of hearing until 2 to 3 months after the operation, as we have stated in the preceding pages. The fascia is also more susceptible to infection than skin flap particularly to infection from outside. We have experienced a few cases of infection of the fascia, which, however, was all arrested in the early stage of superficial necrosis with the help of antibiotics and did not lead to perforation. This is why we leave pieces of gelatin sponge moistened with antibiotic solution in the ear canal for 2 weeks or so.

Admitting some disadvantages of meatotympanoplasty we are still considering it worth while to present our experiences in meatotympanoplasty because the method is superior to classical tympanoplasty in regards to the improvement of hearing as well as easy postoperative care. Even if the hearing gain attained by the method might not be of significant magnitude, our efforts should always be directed to the benefit of patients.

The history of tympanoplasty is short and there is a long way to go before 100% success can be attained. We Japanese otologists have many more cases for tympanoplasty than Europeans and Americans, and we feel keenly the necessity for further research in this field.

#### ZUSAMMENFASSUNG

Der Verfasser hat einige Erfahrungen von „Meatotympanoplasty“ d. h. der tympanoplastischen Operation unter die Erhaltung des äusseren Gehörganges berichtet. Die Operations-Techniken bestehen aus Ablösung der äusseren Gehörgangshaut als gestiehltes Röhrchen aus dem äusseren Gehörgangsknochen, nötigenfalls retrograde Entfernung der äusseren Gehörgangsbrücke, Beseitigung des krankhaften Teils in den mastoidalen Zellen durch den retroauricularen Eingriff, Wiederherstellung des Schalleitungs-Systemes im mittleren Ohr. Neubild-

## EAR MALFORMATIONS IN CLEIDOCRANIAL DYSOSTOSIS

M. Foss

*From the University ENT-clinic Högshospitalet C Copenhagen Denmark*

A case is reported of a 12-year-old girl with familial cleidocranial dysostosis, pronounced ear malformations, and a conductive hearing loss. The right auricle was small and deformed and both external ear canals were treble. Radiograms of the temporal bones showed dense calcification, atretic middle ears, and malformations of the auditory ossicles.

More than 600 cases of cleidocranial dysostosis have been described since the publication by Martin in 1705. Schreihauer (1871) and Marie & Sainton (1897) have had their names associated with the syndrome, but today the more descriptive name cleidocranial dysostosis has been widely accepted.

The etiology of the disease is unknown but seems to be connected with a defect in the parental germ plasma as first suggested by Hultkrantz (1908). The majority of cases appear on a hereditary background but many examples of spontaneous, isolated occurrence have been given. The disease may be transmitted in a few generations and then disappear in the following generations (Witkop-Oosterwijk, 1957). The variety and inconstancy of the skeletal anomalies are prominent features of the syndrome.

The combination of hypoplasia of the clavicles and brachycephalia led to the assumption that bones of membranous origin were primarily affected (Fitchel, 1920). Later investigations have demonstrated that multiple, developmental malformations of the whole skeleton are usually present. Besides hypoplasia of the clavicles and delayed closure of the cranial sutures and fontanelles, reports have been given of prognathism, irregularities in dentition, structural abnormalities in vertebrae, sacrum, pelvis, femora, scapula, metacarpals, metatarsals and phalanges (Hallala & Taskinen, 1962). A recent survey was given by Gorlin & Pindborg (1964).

Although a few cases of mental disease have been reported (Ellgore & Lasker 1946) no constant relationship to the dysostosis has been established.

In the present context the cranial deformities are of special interest. The skull is large and brachycephalic with pronounced frontal and parietal bossing. Due to delayed closure of the sutures, the fontanelles usually remain open and numerous Wormian bodies are formed. The bones of the face are underdeveloped except for the mandible which is long and prognathous. This disproportion of the skull and face in connection with the bulbous body and drooping shoulders makes the general appearance almost pathognomonic. The palate is narrow and highly arched and may have a

- MONIMOTO, M 1962: The choice in tympanoplasty particularly from the standpoint of the treatment of otitis media. *Otologia* (Tokyo) 34 89
- MUTA, M 1964 Fascia graft tympanoplasty without mastoplastic procedure—a new mastoid obliteratory technique *Jap J Otol Tokyo* 67 128.
- MYER, D 1960 Anterior postauricular technique for the treatment of chronic otitis media and mastoiditis *Laryngoscope* 70 78
- NAITO, T., 1961 Some devices in tympanoplasty *O.R.L. Tokyo* 4 250
- 1963 Technical problem in tympanoplasty *Proc. of 16th Gener. Assen. Jap. Med. Cong* 7 783.
- 1964 Tympanoplasty—the problem of the management of mastoid cavity and mastoplastic *Otologia* (Fukuoka) 10 6.
- NISHIMATA, H 1962 Comparison of radical mastoidectomy and bridgemenet of middle ear cavity *Otologia* (Tokyo) 34 867
- ONCHI, Y 1962 The path of conduction after tympanoplasty *Otologia* (Tokyo) 34 537
- OLCHI, J 1960 The mastoplastic method adopted at the otolaryngological department of Fukushima Prefectural Medical University *Otologia* (Tokyo) 32 975
- PALVA, T 1963 Surgery of chronic ear without cavity *Arch Otolaryng* (Chic) 370.
- PORTMANN, M 1963 Tympanoplasty *Arch Otolaryng* (Chic.) 78 2.
- RUYS, J S 1962 Problèmes posés par l'otite chronique dans les tympanoplasties. *R. A. Portmann* 83 114
- SCHNEK, I M 1963: Tympanoplasty—a modification in technique. *Arch. Otolaryng.* (Chic.) 77 87
- SHAMBAUGH, G E, JR, 1962 By pass surgical techniques in stapes surgery *Arch Otolaryng* (Chic) 76 542.
- TAKAHARA, S., 1959 Comparison of utility between pedicle flap and free graft in tympanoplasty *Jap J Otol Tokyo* 62, Suppl. 130
- WOLLSTEIN, H 1952: Funktionell Operationen im Mittelohr mit Hilfe des Freien Spallappen Transplantates. *Arch Ohren Nas. Kehlkopfheilk.*, 161 422.
- 1960 Techniques of tympanoplasty I, II, III *Arch Otolaryng* (Chic) 71 424.
- YAMAMOTO, K., 1962: Problems on the nutrition of the skin flap of tympanoplasty and the recipient base *Otologia* (Tokyo) 34 847
- ZÖLLNER, F 1951: Die bisherige Ergebnisse der Schallisolationsuntersuchung n. *Arch Ohr Nas Kehlkopfheilk* 159 358.

Dept. of Otolaryngology Osaka University  
 Medical School Fukushima-ku Osaka,  
 Japan

Received August 20 1967



FIG. 2. The patient's mother (a) her twin sister (b) and her younger sister (c)

she had been receiving orthodontic treatment for some time. Measuring 142 cm and weighing 37.5 kg, she was considerably smaller than the normal average for her age (158 cm/47 kg)

The familial occurrence was evident as the patient showed a striking similarity to her mother her twin sister and a younger sister (Fig. 2 a b c). On the other hand, her grandparents were normal and to the mother's knowledge there were no other cases of cleidocranial dysostosis in the family.

A complete radiological examination showed all the typical cranial deformities mentioned above. The clavicles were hypoplastic having two small ossification centers each. A spina bifida was found from vertebra C1-T5 and vertebra L5 was also bifid. The pubic bone was incompletely ossified, but the long tubular bones as well as hands and feet were normal.

There was no history or symptoms of intellectual or emotional disturb-



FIG. 3. Frontal (a) lateral (b) and posterior (c) view of the patient's right ear.



FIG. 1 The patient's general appearance was typical of cleidocranial dysostosis.

submucous or complete palatal cleft. Delayed and irregular dentition and supernumerary teeth are usually found. The base of the skull is small, often with incompletely ossified symphyses. The short longitudinal diameter causes extensive arching of brow and occiput. The small width of the cranial base makes the squamous portions of the temporal bones sit in a diagonal position, so that the mastoid processes are displaced in a medial direction and lie in a deeper location than normal. The foramen magnum is large and turned forward, its level passing through the glabella. The clivus is short with an abrupt decline (Hultkrantz, 1908).

In view of these pronounced cranial deformities it would hardly be surprising to find malformations of the petrous portions of the temporal bones with or without functional defects. Nevertheless, no such examples can be found in the extensive literature on this subject and a thorough auditory and vestibular examination does not seem to have been carried out in any of the reported cases. The following example of pronounced ear malformations in a patient with cleidocranial dysostosis is therefore presented.

#### *Case Report*

A 13-year-old girl was admitted to our department with bilateral atresia of the external acoustic meatus and impaired hearing. Her general appearance was typical of cleidocranial dysostosis (Fig. 1) but also typically her physical condition had not called for any previous medical attention while



FIG. 3. Ordinary frontal (a) and lateral (b) radiogram of the cranium.

be identified. The inner ear was normal and the internal acoustic meatus of a normal caliber.

On the left side the external meatus was only slightly diminished but the middle ear was almost as small as on the right side. The ossicles on the left side were no smaller than normal but the handle of the malleus and the long process of the incus could be identified as small prominences. The inner ear and the internal meatus were normal.

An ordinary radiogram of the cranium (Fig. 3a & b) as well as the tomograms mentioned above showed two characteristic findings. On both sides the mastoid cells were absent so that the petrous portions appeared densely calcified. Fig. 3a also shows the typical slope of the two temporal bones.

Pure tone audiometry showed a bilateral conductive hearing loss (Fig. 6). The speech reception threshold was 4 dB on the right side and 20 on the left. By means of acoustic impedance examination the middle ear pressure was found to be normal on the left side and a middle ear reflex was present. On the right side the ear drum impedance remained unchanged with variation of the pressure in the ear canal as evidence of accumulation of fluid in the middle ear.

Caloric-estibular tests were normal.

From the dental examination it was concluded that the maxilla was underdeveloped with a highly arched, narrow palate while the mandible was normal for the age. The dentition was delayed but there were no supernumerary teeth.

The patient's two sisters and their mother showed the radiological find-

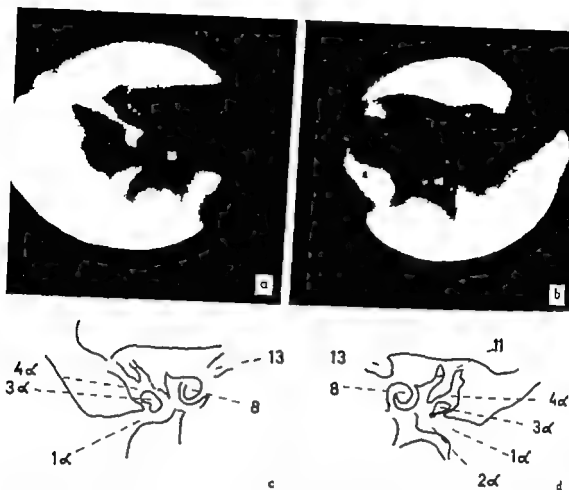


FIG. 4 Frontal tomogram of the right (a) and left (b) ear. Nomenclature of the diagrams (right, d left) 1 External acoustic meatus 2, tympanic cavity 3, auditory ossicles 4 mastoid antrum 8, cochlea 11 superior semicircular canal 13, internal acoustic meatus. Alpha, pathological change (e.g. deformed ossicle, atresia etc.)

ances and the patient seemed socially well adjusted despite her cosmetic handicap. Sexually, she was normally developed and had had regular menstruation for almost one year.

The right auricle was small, coneshaped and turned forward at an angle of 90° with the side of the head (Fig. 3a, b, c). The right external meatus was too narrow for a satisfactory otoscopy but with an operating microscope the tympanic membrane could be seen. It was much smaller than normal and no landmarks could be identified.

The left auricle was normal and the diameter of the left external meatus only slightly under average. The tympanic membrane showed an unusual protrusion of the handle and lateral process of the malleus which was also located close to the posterior metal wall.

On a frontal tomography (Fig. 4a, b, c, d) the right external meatus appeared narrow and the middle ear smaller than normal. The ossicles seemed to form a small irregular bone condensation in the attic and neither the handle of the malleus nor the long process of the incus could



- II TARANTZ, J. W. 1903: Über Dysostosis cleido-cranialis. Longenitale kombinirt Schädels und Schlüsselbeinabnormalien. *Z Morphol. Anthropol* 11 235
- KALLIALA, E., and TARANTZ, P. J. 1962 Cleidocranial dysostosis. Report of six typical cases and one typical case. *Oral Surg* 15, 868.
- KILGORE, S., and LARSEN, G. W. 1916 Cleidocranial dysostosis with psychosis. *Arch Genet Psychiat* 16, 401
- MARIE, P. and SAUTOY, P. 1887 Observation d'hydrocéphalie héréditaire père et fils par suite du développement du crâne et du cerveau. *Bull Mém Soc Méd. Hép. Paris*, 13 704.
- MARTY, M. 1765 3 déplacement naturel de la clavicule. *J Méd Chir Pharmacol* 21, 454.
- SCHMIDTKE, G. 1871 Kombination rudimentärer Schlüsselbeine mit Anomalien des Schädels beim erwachsenen Menschen. *Allg Wl Med. Ztschr* 16, 292.
- WITKOP-CHERNIAK, G. A. 1937 Contribution to the study of the inheritance of dysostosis cleidocranialis. *Acta Gd H* 7 222.

The ENT-clinic Rigshospitalet  
Copenhagen, Denmark

Received July 1 1968

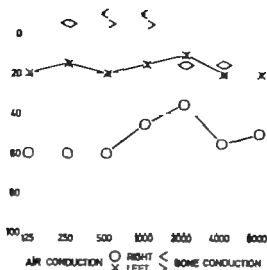


FIG. 6 Pure tone audiogram on deaf lion

ings typical of cleidocranial dysostosis but they had no middle ear malformations and otoscopy and hearing were normal

The serous otitis on the patient's right ear responded promptly to insufflation and administration of ephedrine nasal drops with a subsequent hearing improvement of almost 20 dB

In view of the temporal bone malformations described above the remaining hearing loss seemed only moderate. Consequently a surgical attempt on further hearing improvement did not seem advisable

A plastic operation on the right auricle was planned but for practical reasons it was postponed.

#### ACKNOWLEDGMENT

The author wishes to express his gratitude to Dr H. Røvsing, the Radiological Department, Rigshospitalet for providing the radiograms and diagrams.

#### ZUSAMMENFASSUNG

Bei einem 13-jährigen Mädchen mit Dysostosis Cleidocranialis wurde ein konduktiver Hörverlust und schwere Ohrenmissbildungen gefunden. Die rechte Aurikula war klein und missgebildet und Meatus akustikus externus war auf beiden Seiten atretisch. Röntgenaufnahmen der Felsenbeine zeigten dichte Sklerose atretische Mittelohre und Missbildung der Gehörknöchelchen.

#### REFERENCES

- FITCHET H. M. 1929 Cleidocranial dysostosis. Hereditary and familial. *J. Bone Joint Surg. (Brit.)* 11: 838.  
 GORLIX J. G. and PANDRONI, J. J. 1964 *Syndromes of the head and neck* pp. 138-142. MacGraw Hill

among others have performed fundamental anatomical investigations, many questions will remain which cannot be answered before we know more about the anatomy of the system.

What is it like compared to other known species, man or cat, and even more interesting, compared to the whales, which also employ echolocation?

The present investigation tries to add to our knowledge by applying two methods recently developed for investigation of the cochlea and the cochlear nuclei.

### MATERIAL AND METHODS

The material consists of 20 specimens of the microchiropterae *Plecotus auritus* collected from an old church tower in Norway. They were anesthetized by ether until soundly asleep and then decapitated. Ten animals were used for preparation of the cochlea, the others for preparation of the brain and the VIIIth nerve. For the preparation of the cochlea the osmium tetroxide fixation and the surface preparation technique described by Engström *et al.* (1966) was applied. The brains were fixed in 10% Formaline, sectioned in 10 microns and stained with Thionine. The method described by the author (Hall, 1964 and 1967) was then applied for counting the number of cells and for computing the volume of the cochlear nuclei.

The part of the medulla containing the cochlear nuclei was sectioned in 10 micron sections, every 10th section was magnified and the outlines of the nuclei drawn on paper employing a Baurch & Lomb projection apparatus. Then their areas were determined using a planimeter and the volume calculated by simple mathematics.

### OBSERVATIONS

Although this paper primarily deals with the cochlea and the cochlear nuclei, some data collected concerning the external parts of their sound perceiving system might also interest the reader. As indicated in their names, the pinnae of the *Plecotus auritus* were enormously large, they measured up to 5 cm in length, whereas the body including the head, was only 7 cm long (Fig. 1). The width of the skull was 9 mm, and the bullae occupied 3 mm on either side (Fig. 2).

The oval ear-drum stretched out within the bulla measured  $2 \times 3$  mm, thus occupying one-third of the transverse diameter of the head (the ear drum in man measures  $9 \times 8$  mm).

The ossicles of the middle ear are situated as in other mammals, but some details may be significant for the perception of higher frequencies. The head of the malleus is comparatively great, its shaft broad, more or less formed like a plate (Fig. 3).

The long leg of the incus is gracile, there is a distinct processus lenticularis, articulating with a small, thin and fragile stapes whose crurae as in homo, are inward concave.

## THE COCHLEA AND THE COCHLEAR NUCLEI IN THE BAT

J G HALL

*From the Anatomical Institute and the ENT Department University of Oslo, Norway*

The outer and inner ears and the cochlear nuclei in the bat *Plecotus auritus* are described. The author has collected 20 specimens of this animal, whereof 10 were used for preparation of the cochlea, applying the method of H Engström. In the other half the cochlear nuclei were prepared following the method described by the author in 1964. The volume of the cochlear nuclei and their number of nerve cells are computed and the distribution of the primary nerve fibres is described. The primary auditory centers in the bat are compared with those of the cat, the whales and homo.

Some of the nocturnal carnivorous microchiroptera are wholly dependent upon their hearing as echolocation provides their means for the location of obstacles and prey (Spallanzani 1794, Hartridge, 1920, Galambos and Griffin 1940, Dijkgraaf 1946, Møhrén, 1952, Griffin 1958, Grinnell 1958). The echoes result from cries of high intensity produced in a highly specialized larynx (Pye 1961).

The intensity of their calls has been measured 10 cm in front of their mouth and may at that point reach 60 Dyn or approximately 110 dB. They usually employ a frequency range from 20-120 kilocycles/sec, thus practically inaudible to man. The frequency of the call also in some species varies from high to low, declining about one octave during each call. When not flying, they emit only a few cries, about 8/min, but during flight or prey catching they may emit up to 200 cries/sec. As they are able to detect their own echoes even in caves where several hundred bats are shrieking at the same time and even in white noise covering their own spectrum, a highly specialized discriminative system must be involved. Pye (1960) has proposed that they must be listening to the beat note occurring between the call and the echo.

Griffin (1958) has also shown in *Vespertilionidae* that they employ different frequency modulations in each cry and that the number of cries/sec may vary considerably, increasing when nearing an object. The highly specialized forms of the outer ears, especially in *Vespertilionidae* and the quick movements of the outer ears (*Rhinolophidae*) certainly also represent suitable means for detecting the echo. The investigations cited above have thrown some light upon the function of the extraordinary auditory system in bats. However, although Poljak (1946) and Reysenbach de Huan (1958)

among others have performed fundamental anatomical investigations, many questions still remain which cannot be answered before we know more about the anatomy of the system.

What is it like, compared to other known species, man or cat, and even more interesting, compared to the whales, which also employ echolocation.

The present investigation tries in addition to our knowledge by applying two methods recently developed for investigation of the cochlea and the cochlear nuclei.

### MATERIAL AND METHODS

The material consists of 20 specimens of the microchiropterae *Plecotus auritus* collected from an old church tower in Norway. They were anesthetized by ether until soundly asleep and then decapitated. Ten animals were used for preparation of the cochlea, the others for preparation of the brain and the VIIIth nerve. For the preparation of the cochlea the osmium tetroxide fixation and the surface preparation technique described by Engström *et al.* (1966) was applied. The brains were fixed in 10% Formaline, sectioned in 10 microns and stained with Thionin. The method described by the author (Hall, 1964 and 1967) was then applied for counting the number of cells and for computing the volume of the cochlear nuclei.

The part of the medulla containing the cochlear nuclei was sectioned in 10 micron sections, every 10th section was magnified and the outlines of the nuclei drawn on paper employing a Bausch & Lomb projection apparatus. Then their areas were determined using a planimeter and the volume calculated by simple mathematics.

### OBSERVATIONS

Although this paper primarily deals with the cochlea and the cochlear nuclei, some data collected concerning the external parts of their sound perceiving system might also interest the reader. As indicated in their names, the pinnae of the *Plecotus auritus* were enormously large; they measured up to 2 cm in length, whereas the body including the head, was only 7 cm long (Fig. 1). The width of the skull was 9 mm, and the bullae occupied 3 mm on either side (Fig. 2).

The nasal ear-drum stretched out within the bulla measured  $2 \times 3$  mm, thus occupying one-third of the transverse diameter of the head (the ear drum in man measures  $9 \times 8$  mm).

The ossicles of the middle ear are situated as in other mammals, but some details may be significant for the perception of higher frequencies. The head of the malleus is comparatively great, its shaft broad, more or less formed like a plate (Fig. 3).

The long leg of the incus is gracile; there is a distinct processus lenticularis, articulating with a small, thin and fragile stapes whose crurae, as in man, are inward concave.

## THE COCHLEA AND THE COCHLEAR NUCLEI IN THE BAT

J G HALL

*From the Anatomical Institute and the E.N.T. Department University of Oslo, Norway*

The outer and inner ears and the cochlear nuclei in the bat *Plecotus auritus* are described. The author has collected 20 specimens of this animal whereof 10 were used for preparation of the cochlea, applying the method of H Engström. In the other half the cochlear nuclei were prepared following the method described by the author in 1964. The volume of the cochlear nuclei and their number of nerve cells are computed and the distribution of the primary nerve fibres is described. The primary auditory centers in the bat are compared with those of the cat, the whales and homo.

Some of the nocturnal carnivorous microchiroptera are wholly dependent upon their hearing as echolocation provides their means for the location of obstacles and prey (Spallanzani, 1794 Hartridge 1920 Galambos and Griffin, 1940 Dijkgraaf 1946 Mjöres 1952 Griffin, 1958 Grinnell 1958). The echoes result from cries of high intensity produced in a highly specialized larynx (Pye, 1961).

The intensity of their calls has been measured 10 cm in front of their mouth and may at that point reach 60 Dyn, or approximately 110 dB. They usually employ a frequency range from 20-120 kilocycles/sec, thus practically inaudible to man. The frequency of the call also in some species vary from high to low declining about one octave during each call. When not flying, they emit only a few cries, about 8/min, but during flight or prey catching they may emit up to 200 cries/sec. As they are able to detect their own echoes even in caves where several hundred bats are shrieking at the same time and even in white noise covering their own spectrae a highly specialized discriminative system must be involved. Pye (1960) has proposed that they must be listening to the beat note occurring between the call and the echo.

Griffin (1958) has also shown in *Vespertilionidae* that they employ different frequency modulations in each cry and that the number of cries/sec may vary considerably increasing when nearing an object. The highly specialized forms of the outer ears, especially in *Vespertilionidae* and the quick movements of the outer ears (*Rhinolophidae*) certainly also represent suitable means for detecting the echo. The investigations cited above have thrown some light upon the function of the extraordinary auditory system in bats. However although Poljak (1920) and Reysenbach de Haan (1958)



FIG. 2. The middle ear ossicles. Note the flat, plover-like shape of the hammer. 25.

brane 6.3 mm, the latest number found by measuring on a photograph after removing the basilar membrane in sections (Fig. 4). The length of the basilar membrane in man is 30–35 mm (Bredberg, 1968).

The organ of Corti is built as in other mammals, with three distinct rows of outer hair cells and one row of the inner hair cells. The outer hair cells were long and slender and the inner hair cells short, plump, flasklike, with a great round nucleus. A counting under the microscope showed that 50 of the inner hair cells occupied the same space as 60 in one of the outer rows (Fig. 5).

The hairs, when seen from the surface, show the same "W" form as in man and cat (Fig. 6).



FIG. 4. Preparation of the cochlear membrane. 26.



FIG. 1 *Plecotus auritus*, the most common bat in Norway. Note the long ears.

The cochlea has  $2\frac{1}{4}$  coils, whereof the basal one is by far the greatest. Its diameter measured with a micrometer in the microscope was 2.33 mm (in man 7 mm) whereas the upper coil measured only 1.27 mm.

The height of the cochlea was 2.7 mm and the length of its basilar mem-



FIG. 2 Upper jaw and cochlea. On the right side, the bulla is removed and the cochlea is visible. On the left side the bulla is removed, and the cochlea is inside the skull.





FIG. 2. The middle ear ossicles. Not the flat, pear-like shape of the hammer. 25

brane 6.3 mm, the latest number found by measuring on a photograph after removing the basilar membrane in sections (Fig. 4). The length of the basilar membrane in man is 30-35 mm (Bredberg, 1968).

The organ of Corti is built as in other mammals, with three distinct rows of outer hair cells and one row of the inner hair cells. The outer hair cells are long and slender and the inner hair cells short, plump, flasklike with great round nucleus. A counting under the microscope showed that 50 of the inner hair cells occupied the same space as 60 in one of the outer rows (Fig. 5).

The hairs, when seen from the surface, show the same W form as in man and cat (Fig. 6).



FIG. 4. Preparation of the cochlear membrane. 30



FIG. 1 *Plecotus auritus*, the most common bat in Norway with the long ears.

The cochlea has  $2\frac{1}{4}$  coils, whereof the basal one is by far the greatest. Its diameter measured with a micrometer in the microscope was 2.33 mm (in man 7 mm) whereas the upper coil measured only 1.27 mm.

The height of the cochlea was 2.7 mm and the length of its basilar mem-



FIG. 2. Upper jaw and cochlea. On the right side, the bulla and the eardrum (tympanic membrane) are visible. On the left side the bulla is removed, and the cochlea is laid out.



FIG. 1 The cochlea, the VIIIth nerve and the cochlear nuclei.  $\times 60$

parts of the nuclei are seen in square sections in the Figs. 10 and 11. Fig. 10 shows the dorsal (top of picture) and the posteroventral nucleus, and Fig. 11 the anteroventral (top) and the posteroventral nucleus.

The number of nerve cells counted, compared to other species are seen in Table 2.

The dorsal cochlear nucleus was counted separately and the reason for this was our present interest in the role of this nucleus (Hall, 1964; Oxen & Jansen, 1965) in different species (see Discussion).



FIG 5



FIG 6

FIG 5 The outline of the three rows of inner hair cells, and the single row of outer hair cells.  $\times 1000$

FIG 6 The W<sup>sh</sup> formed by the tip of the hairs on top of the hair cells. 1250

The spiral ganglion situated in the modiolus, contain great oval cells, with their nuclei centrally placed. The space separating this ganglion from the cochlear nuclei is narrow (Fig 7) which was the reason why a preparation of this short VIIIth nerve for a fiber count, was not successful.

Fig 8 shows the usual square section of the organ of Corti where especially the lamina spiralis ossea secundaria is remarkable (Reysenbach de Haan 1958).

The cochlear nuclei are situated in the lateral part of the medulla at the lateral corners of the fossa rhomboidea. They bulge out like an extramedullary pouch on each side, occupying a substantial part of the brain stem.

The following facts about the cochlear nuclei were determined. The complex of the cochlear nuclei measured 2 mm in length on average, forming a spindle, whose diameter in its widest part was 0.5 mm. The cubic content was  $0.6 \text{ mm}^3$  which represents one fourth of that in the cat.

The cochlear nuclei can be divided into three main sections, as in cat and man the anteroventral, the posteroventral and the dorsal nucleus (Fig 9). Fig 9 shows drawings of square sections of the cochlear nuclei which is used for computing the volume and the number of nerve cells. The nerve enters rostroventrally and the nerve root continues dorsocaudally between the antero- and the posteroventral nucleus.

The volume of the cochlear nuclei are seen in Table 1 compared to other species (Hall 1964 and 1967). It is seen in this table, that the cochlear nuclei in bats are far more voluminous than in the others, compared to the size of the animals. They constitute about one third of those in a kitten, and one sixteenth of those in man which is a substantial magnitude for an animal weighing only 10–12 g. Representative pictures of the three different



FIG. 10.



FIG. 11

FIG. 10. The dorsal and the posterovenral nucleus. 240. Separation line tipped.

FIG. 11. The anteroventral and the posterovenral nucleus. 240. Separation line stippled.

The cochlear nucleus in the bat contains on average 40 000 cells, whereof 7000 cells belong to the dorsal cochlear nucleus. The layer of granular cells covering the cochlear nucleus (especially the dorsal one) was not counted, only the nerve cells containing a nucleus surrounded by a distinct cytoplasm.

As it is of great interest to know also the ramification of the nerve fibres distributed upon the cells of the cochlear nuclei, this point was studied separately.

A sagittal section, showing the ramifications of the nerve inside the nuclei is shown in Fig. 12. (Osen, 1966) The ascending fibres, to the left in the

TABLE 1. Volume of the cochlear nuclei (in mm<sup>3</sup>)

Bat	Kitten	Human	Porpoise	Fin whale	White whale
0.6	2	18	80-100	205	320



FIG. 8. Tb

THE COCHLEAR NUCLEI IN TH

FIG. 9. Schematic drawing of square drawn



FIG. 10



FIG. 11

FIG. 10. The dorsal and the posterodorsal nuclei. 240. Separation line stippled.

FIG. 11. The anteroventral and the posteroventral nuclei. 240. Separation line stippled.

The cochlear nucleus in the bat contains on average 40,000 cells, whereof 7000 cells belong to the dorsal cochlear nucleus. The layer of granular cells covering the cochlear nucleus (especially the dorsal one) was not counted, only the nerve cells containing a nucleus surrounded by a distinct cytoplasm.

As it is of great interest to know also the ramification of the nerve fibres distributed upon the cells of the cochlear nuclei, this point was studied separately.

A sagittal section, showing the ramifications of the nerve inside the nuclei is shown in Fig. 12. (Osen, 1966) The ascending fibres, to the left in the

TABLE 1. Volume of the cochlear nuclei (in mm<sup>3</sup>)

Bat	Kitten	Human	Porpoise	Fin whale	White whale
0.6	2	10	60-100	205	320



FIG 8. The organ of Corti

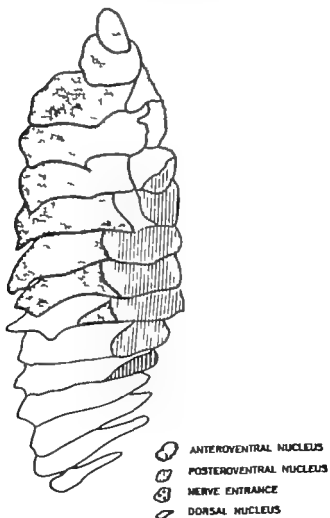
THE COCHLEAR NUCLEI IN THE BAT *PLECOTUS AURITUS*

FIG 9. Schematic drawing of square section of the cochlear nuclei. Each 10th slide drawn.



## DISCUSSION

The outer ears in bats are extremely well developed, and also their middle and inner ears are of considerable size compared to the size of the animal. As to the general anatomical structure however this is similar to that of other mammals. The outer ears, measuring more than  $\frac{2}{3}$  of the body and the ear-drums, whose size is about  $\frac{1}{2}$  of those in man, show that we are dealing with a highly specialized system. The cochlea also has considerable dimensions, but the most remarkable feature was the size of the cochlear nuclei. They were not only voluminous, but also contained 40 000 nerve cells, which is about 40% of the number of nerve cells in man. As their whole existence is based upon echolocation and they possess no obvious communication, all these cells must be mainly employed in the perceiving of echoes. 7000 or about 17% of the cells were situated in the dorsal cochlear nucleus. Comparing man, cat and different whale species it is seen, that in species which apply high frequencies or supersonics for the finding of prey echolocation or perhaps communication (whales) the number of nerve cells in the dorsal cochlear nucleus is diminishing, while in man and kitten this part occupies a substantial part of the complex. Thus it is reasonable to assume, that the transmission of the high frequencies in the echolocating species is more specifically carried out by the ventral nucleus, which is the dominating one, especially in whales, but also in bats, both concerning the volume and the number of nerve cells. The dorsal cochlear nucleus, however is rudimentary in whales, but somewhat more pronounced in the bats. In the dorsal cochlear nucleus the cells are arranged in rows or layers, somewhat like a cortical arrangement, but this is not so prominent as in cat or man. The nerve fibres reach the cells of the dorsal nucleus through the posterior part of the posteroventral nucleus (Fig. 12) where they are twisted 180° (Osen, 1966). This point provides the anatomical basis for the findings of Rose *et al.* (1959) who have shown that the low frequency carrying fibres are situated ventrally and the high-frequency carrying fibres dorsally in each part of the complex. To achieve this the fibres converge when passing through the posteroventral nucleus, and then twist 180°.

This study of structures and measurements in bats has revealed large dimensions both of the cochlea and of the cochlear nuclei compared to other species. Also the complexity of the cochlear nuclei in the bats points to a highly developed system supervising their supersonic abilities.

## ZUSAMMENFASSUNG

Das äussere und innere Ohr der Fledermäuse *Plecotus Auritus*, sowie die Cochlearkerne derselben werden beschrieben. Der Verfasser hat 20 Exemplare gesammelt, in welchen die Cochleae von 10 Exemplaren nach der Methode von



FIG. 12. The ramifications of the eighth nerve in the cochlea. 11 120

picture, spread out fanlike in the anteroventral nucleus. The descending ones however converge in the caudal part of the posteroventral nucleus where they are twisted 180 degrees, and then also these spread out fanlike.

TABLE 2 Number of nerve cells in the cochlear nuclei of different species

Species	N. 8 cell		
	Total	Dors. nucl.	Dorsal (total)
Bat	40,000	7,000	17
Kitten	100,000	29,000	29
Homo	90,000	30,000	32
Porpoise	600,000	40,000	8.0
Whit whale	1,650,000	24,000	1.5

## SOME EXPERIENCES WITH THREE HUNDRED STAPEDECTOMISED PATIENTS

L. E. FLOBERG, B. IYSSAM and T. LUNDBORU

*From the E.N.T. Clinic and Audiological Unit Södersjukhuset and Hörstikklåken  
Stockholm Sweden*

At the E.N.T. Clinic, Södersjukhuset, stapedectomy (with vein graft in the oval window and application of polyethylene tube on incus) has become the method of choice for 500 operated otosclerosis patients 1962-1967. The results from 300 consecutive stapedectomies in 1967 are discussed. The experience of the actual case material reveals that, for instance, the age of patients at admission is immaterial and that many audiometrically classified C- and D-cases seem to have very good prognostic possibilities. The postoperative hearing results—according to the definition of closed gap and a average improvement in the middle frequencies—are good—ca. 93% success as in many other series. Postoperative deafness has appeared in 4 cases out of 300. From the point of view of hearing rehabilitation the results have been especially satisfactory. 90% of the patients could dispense their hearing aids or change from bodyborne to headborne apparatus thus this factor is the essential indication for an eventual operation. There is needed a continuous postoperative hearing control, especially concerning "late" cochlear lesion. Uniform definitions and classifications of case materials, for instance common use of a A.D.P.-system, will probably give a more certain basis for prognostic evaluation and possibly indicate changes in operative technique.

During the period 1962-1967 more than 500 otosclerosis patients have been operated upon in the E.N.T. clinic of Södersjukhuset. In the majority of cases stapedectomy has been performed with vein graft in the oval window niche and application of polyethylene tube on processus longus incudis. During the whole period the original Rosen-technique has never been used, nor has stapediolysis been performed directly on the footplate except in 3 cases with very advanced niche otosclerosis. On the other hand, Fowler's technique i.e. stapediolysis with anterior crurotomy and partial stapedectomy has been used in quite a number of cases. Gaining experience that this kind of technique too often causesankylosis—which is well known—this type of operation has been abandoned gradually. It has been performed only in cases with "ideal circumstances" i.e. mostly with restricted otosclerotic areas around the anterior crus, which can be easily removed, with the rest of the footplate freely movable, with intact incudo-stapedial

H Engström präpariert sind, von den letzteren 10 sind die Cochlearkerne nach der von dem Verfasser in 1964 beschriebenen Methode präpariert worden weiter sind ihre Volumen sowie ihre Nerven Zellenanzahl berechnet, und die Verteilung der primären Fibern wird beschrieben

Die Verhältnisse dieser Bildungen in der *Plecotus Auritus* werden mit anderen Arten (Katzen Walen und Homo) verglichen

## REFERENCES

- BRODKEG, G 1968 Cellular pattern and nerve supply of the human organ of Corti. *Acta Otolaryng* (Stockh.) Suppl 236
- DUKORRAAF S 1946 Die Sinneswelt der Fledermäuse *Experimentia*, 2 438.
- ENGSTRÖM, H, ABER, H W., and ANDERSON, A 1966 *Structural pattern of the organ of Corti*, Almqvist & Wiksell, Stockholm.
- GALAMBOS, R and GRIFFIN D R 1940 The supersonic cries of bats. *Ann Ent Rec* 8 35.
- GRIFFIN, D R 1942 Obstacle avoidance by flying bats, the cries of bats. *J Exp Zool* 89 475
- 1958: *Listening in the dark* Yale University Press.
- GRIFFIN, A. D 1958 The sensitivity of echolocation in bats. *The Biological Bulletin* 114 10
- GRIFFIN, A. D and GRIFFIN, V S 1965 Neural correlates of vertical localisation by echo-localising bats *J Physiol* 181 830
- HALL, J G 1964 The cochlea and the cochlear nuclei in neonatal asphyxia *Acta Otolaryng* (Stockh.) Suppl. 191
- 1967 Hearing and primary auditory centres of the whale. *Acta Otolaryng* (Stockh.) Suppl 224 244
- HARTNIDGE, H. 1920 The avoidance of objects by bats in their flights. *J Physiol* 54 54
- MORRIS, F P 1952 Die Ultraschall-Orientierung der Fledermäuse. *Die Naturwissenschaften* 39 273
- OSSEN K. R., 1966: Personal communication
- OSSEN K. R., and JAMES J 1965 The cochlear nuclei in the common porpoise, *Phocoena phocaena*. *J Comp Neurol* 123, 223
- POLJ K, S 1926 Untersuchung an Olfaktorik in der Säugetiere an den mit diesen koordinierten motorischen Apparaten des Hirnstammes. *J Psychol Neurol* (Lpz.) 32 170.
- PIE, A 1966: The *Megachiroptera* and *Vespertilionidae* of the *Microchiroptera*. The structure of the cochlea in *Chiroptera* *J Morph* 118 495
- PIE, J D 1959 60 A theory of echolocation by bats. *Report Inst of Laryngol and Ot* 10/11 114
- 1961 Echolocation by bats. *Endavour* 78, 101
- REINERBACH DE HAAN, F W 1958 Hearing in whales. *Acta Otolaryng* (Stockh.) Suppl 134
- ROSS, J E., GALAMBOS, R and HUANG, J H 1959 Microelectrode studies of the cochlear nuclei in the cat. *Bull Johns Hopk H* p 104 211
- S ALLANKANT, L 1932 *Ope di Lax Spallanti* 1 3 Urico Heopli, Mla

E.N.T. Department Entomology of Ost  
Ola No way

R i d August 30 1968

*Symptomatology*

The clinical facts have been registered on special audiology journals suited for automatic data processing (Lundborg, 1965) and the actual study has been made possible by grants from "Sjura". In this connection only a relevant part of all information, which is available from the data processing, is presented.

*Distribution of Age*

The actual patient material merits special interest from this point of view according to Table 1. If one compares this condition with the indication for fenestration, where an age above the middle age was considered a contra-indicative factor. The distribution of age among the 300 stapedectomised patients reveals first a rather higher average than among fenestrated patients (Skoog, 1953) and also, that a relatively large group of patients—11—is more than 70 years old, in two cases even 80 years old, which demonstrates that a maximum age does not seem to exist, which also has been demonstrated by Andersen & Warrer (1968) and others. When the case material is classified in A-B-C and D-cases (according to Shambaugh) there is another interesting aspect to compare with for instance Skoog's (1953) material of fenestrated patients (Table 4).

In a rather large group of the patients the average of A.C.-level in the middle frequencies was less than 80 dB, in some cases even at the 100 dB level or without any registrable A.C. in certain frequencies. These patients were almost, without exception, old hearing aid cases, where at earlier evaluation indication of fenestration was not considered to exist. In many of these cases there had also been made attempts earlier to perform indirect stapediolysis.

As can be seen from Table 4 the B.C. levels of the case material revealed that almost 1/3 of the cases were D-cases with audiometrically established cochlear lesion with mean of B.C. level less than 35 dB, in some cases even without registrable B.C.-level. Most of the patients were C-cases, a minor group were B- and A-cases.

A.D.P.-registration has used 10 dB steps, why a certain evaluation is necessary when the patients shall be grouped according to Shambaugh. It is, in any case, obvious that on the whole in the actual case material, there is a clear tendency to an average of impaired cochlear function compared with Skoog's (1953) material of fenestration cases. In cases with clearly impaired cochlear function or in cases where this function is very difficult to evaluate the performance has naturally been done as an attempt to get some utilization of the least usable ear often on the patient's eager request. Another diagnostic difficulty encountered in such cases, is to evaluate if and how the cochlear lesion is progredient. When it has been possible to repeat the examinations on the same patient over a fairly long period and

joint and with good subjective improvement of hearing. This changed attitude towards the partial stapedectomy technique is apparent for instance from the fact that during 1962-44 stapediolytic were performed, but during 1966 only 3. Thus stapedectomy has become the method of choice and stapediolytic with partial stapedectomy has—as mentioned—been performed only exceptionally under special circumstances. Therefore there is little reason to discuss results and prognosis in stapediolytic, because these facts are well known. Because the patients in a large group of the cases were fairly aged and using hearing aids for many years, and a modified type of polyethylene tube has been used there are special reasons—primarily socio-medical—to discuss the results after stapedectomy in 300 consecutive cases.

The anatomical and histological conditions in the oval window niche with all variations of the otosclerotic processes etc. are well known thus there is no reason to present details in this connection.

### Diagnosis

As a rule the patients have been examined with a test battery comprising pure tone-audiometry, speech-audiometry, Békésy audiometry (Grason Stadler Narrow Band Noise, mod. E 800), Gellé-test and registration of stapedial reflex (Madsen model Z001). The examinations with this test battery give a well known result pattern.

(a) Gap between A.C. and B.C. with types of curve in A.C. mostly revealing rising, flat or gradual downward curves. In a few cases other curve types (Table 3) and with B.C. in varying levels (Table 4) i.e. corresponding to A-B-C and D-cases according to Shambaugh's terminology.

(b) Discrimination scores corresponding to B.C. level.

(c) Békésy audiograms revealing type I Jerger.

(d) Gellé-test negative i.e. revealing only direct or "osseal bone conduction" but not "indirect" or "osseotympanic" bone conduction.

(e) Stapedial reflexes cannot be registered. Only in one case has there been registered normal stapedial reflexes, which—after double check—could be explained at operation by the presence of very thin stapedial crurae in connection with a totally fixed foot plate thus making possible contractions of the stapedial muscle.

Several tests have been used on each patient with the aim of getting consistent results. Practically this routine has been verified by a correct diagnosis in all cases. The evaluation of bone conduction level and cochlear function—as is well known—often reveals difficulties and uncertainty especially in the group of D-cases, for instance in old patients having tinnitus and using an unilateral hearing aid for many years. During these conditions the ear actual for operation at examination may offer special difficulties because of its lack of daily life stimulation.

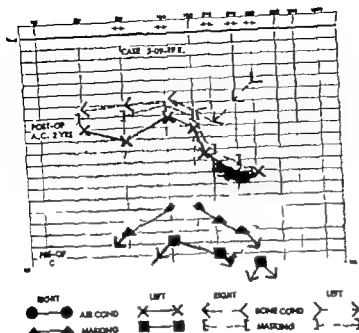


FIG. 2. Case 1913-09-10, E. K. Male patient with body-borne hearing aid with very insufficient communication and social condition (alcoholic). Post-op., using head-borne hearing aid only occasionally; after one year well rehabilitated and socially cooperating.

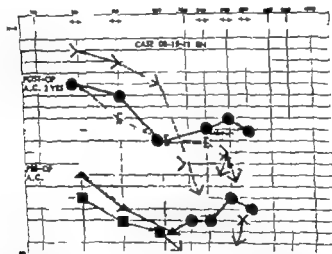


FIG. 3. Case 1903-13-11 E. M. Female patient, foreign born, using body-borne hearing aid, not able to speak after 10 years. Post-op., head-borne hearing aid occasionally. Good linguistic progress.

in the usual window niche, and at a slight pressure on malleus, smoothly following the movements of incus. This is a pre-requisite to get adequate columellar contact with permanent fixation of the prosthesis on incus but without pressure on the long process. It is practically impossible for a

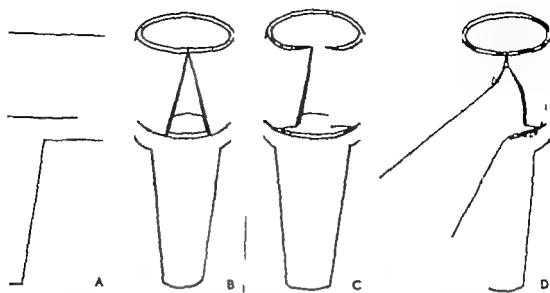


Fig 1 The polyethylene stapes prosthesis. (A) The "collar" of prosthesis is formed by transversal section of the circumference (B) oblique longitudinal incision in the "collar" (C) with the prosthesis introducer in position, the longitudinal opening is widened enough to grip around incudal process (D) after removal of introducer prosthesis remains in a smooth self locking position"

when the B.C. level and discrimination scores have revealed successively deteriorated cochlear function, the performance has been refused. In some cases of this type, without any operative performance a total cochlear lesion has been stated. If some of these cases had been operated upon, with all probability the surgical performance would have been considered responsible for the cochlear lesion. There is reason to believe, that this factor may partly exist in many case materials, but—as far as we are aware—it has not been sufficiently emphasized in the discussion of postoperative cochlear lesions. When the case material has been checked over a sequence of years, it may be possible to get better statistically based rules concerning the relation between audiometrically established cochlear reserve and prognosis. With actual experience, in isolated cases, it seems very difficult to formulate universal prognostic rules.

The stapedectomy performance was a modified Goodhill technique (Goodhill 1961) i.e. removal of the foot plate with a vein graft in the oval window niche and application of a polyethylene tube on processus longus incudis. The prosthesis was cut to suitable length and shape according to Fig 1. To ensure good fixation of the prosthesis to the incudal process without causing simultaneous circulatory disturbance of the process, the prosthesis was shaped to be self locking. According to actual experience these requirements were fulfilled with the type used. The longitudinal oblique incision of the prosthesis collar formed an opening with two wings clutching the incudal process as if in a grip. When the introducer was removed the polyethylene tube was placed in position firmly on incus, well centred



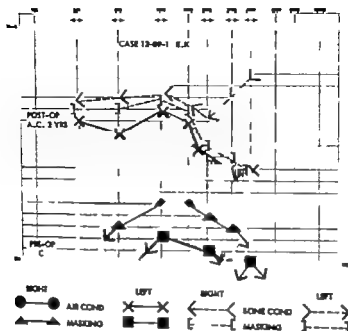


FIG. 2. Case 1912-09-19. E. K. Male patient with bodyborne hearing aid with very insufficient contralateral and social condition (alcoholic). PostOp., using headborne hearing aid only occasionally; after one y. well rehabilitated and socially cooperative.

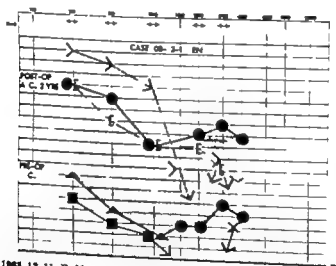


FIG. 3. Case 1903-13-11 E. M. Female patient, foreign born, using body borne hearing aid, not speaking after 10 years. PostOp. headborne hearing aid occasionally. Good qualitative progress.

In the oval window niche, and at a slight pressure on malleus, smoothly following the movements of incus. This is a pre-requisite to get adequate columellar contact with permanent fixation of the prosthesis on incus but without pressure on the long process. It is practically impossible for a

TABLE 1 *Distribution of age in 300 stapedectomised patients*

Age yrs	No. of pts.
> 70	51
60-69	91
51-59	84
41-49	50
< 40	24
Total	300

prosthesis of this type correctly applied on the incudal process, to be dislodged. In our experience this type of tube has never failed in this connection (Silfala, 1965). Gaining good experience with this type of technique we have not found it worthwhile to use any other form of technique (Portmann technique, teflon prosthesis, Schuknecht prosthesis etc.) The idea has principally been—as Portmann (1965) stated at the VIII Int. congress of O.R.L.—“to achieve a permanent opening in the oval window and create a columellar contact between incus and the oval window. How this is performed in detail is a secondary question.” The details in operative technique are well known and need thus not be discussed. Local anesthesia was normally used. In only 10 cases was general anesthesia performed with intubation. The oval window opening was covered with a vein graft from the dorsal part of the hand, gelfoam or other foreign material was not used.

### Results

There have been no complications in form of otitis, labyrinthitis or meningitis in direct connection with operation, which has been related in many other case materials. On the other hand, in several cases there have

TABLE 2 *Air conduction level in 300 stapedectomised patients*

Mean of 500-1000-2000 Hz.

A.C. level, dB	No. of pts.
> 80	41
71-80	44
61-70	67
51-60	91
41-50	48
40	9
Total	300

TABLE 3 *Type of curve in 300 stapedectomised patients*

Type of curve	No. of pts.
Flat	145
Rising	75
Gradual down	33
Marked down	9
Trough	5
Humpback	1
Others	1
No registrabl. A.C.	8
Total	300

TABLE 4. Preoperative bone conduction level in 300 stapedectomised patients

B.C.-level, dB	No. of pts.
Not reg.	8
> 50	19
50-41	40
40-31	84
30-21	113
20-11	34
< 10	3
Total	300

been nose and throat infection shortly after the performance—tonsillitis, sinusitis and in one case also an acute otitis with spontaneous drum rupture—but in no case with simultaneous lesion of the labyrinth. According to our experience the vein graft put in proper position in the oval window niche, thus ensures a good protection of the labyrinth. Special complications temporary facial paralysis four cases liquor drainage in one case, which healed without cochlear lesion.

### Hearing Results

The great majority of patients enjoy excellent hearing postoperatively as is well known from many other case materials. To evaluate the improvement of hearing and the effect of operation, a follow up study for several years is necessary. It is obvious, however that the actual case material reveals a clear tendency concerning good hearing results, thus there seems to be no reason to change the technique used at the moment. The follow up of the cochlear function should decide whether a less radical technique is needed.

TABLE 5. Postoperative hearing results

Patients examined		
6 months	300	Closed air-bone gap 234 Air-bone gap 8 Deaf 4 Missed <sup>a</sup> 4
1 year	292	Closed air-bone gap 233 Air-bone gap 9
2 years	232	Closed air-bone gap 222 Air-bone gap 10

<sup>a</sup> partly decreased by irrelevant disorders, in patients missed way it values addresses the three month control revealed closed air bone gap in all four cases.

From the *hearing rehabilitation* point of view there have been such satisfactory results (i.e. that the patient not need to use a hearing aid or only has to utilize a hearing aid occasionally or can use a headborne hearing aid instead of a bodyborne apparatus) that this positive experience now by the authors—as for instance by Johansen (1967)—is looked upon as the essential operative indication. Because of this a preliminary report would appear useful.

The registration of the postoperative A.C. and B.C. levels has followed Goodhill's system (Goodhill 1961) i.e. firstly to note if there is an air-bone gap or not. In the cases without air-bone gap the postoperative average improvement of hearing in the middle frequencies (500–1000–2000 Hz) varied between 25–30 dB and 50–60 dB. Why an operative elimination of a totally fixed foot plate results so differently may be assumed to depend on the partly unknown factors which determine the variations of Carhart notch.

More than 50% of the patients (152 of 300) had a preoperative A.C. level—mean of middle frequencies—less than 60 dB and therefore needed a bodyborne hearing aid permanently. The other 148 patients mostly used a hearing aid or had a hearing aid prescription at the time of operation. The postoperative hearing gain thus made it possible for 90% of the patients to dispense with the hearing aid used for many years or change from a bodyborne to a headborne apparatus. This mode of amplification for most patients is far preferable thus making it possible, for instance not to stop at call which of course means great relief and benefit (Table 2).

In this connection some of the foreignborn patients merit special interest from the rehabilitation point of view. In four cases (all women) who permanently used bodyborne hearing aids, one could state that the degree of hearing impairment (A.C.-curves in the 70–80 dB level) despite amplification did not permit the learning of Swedish, though there was a linguistic exposition for more than 10 years. The postoperative hearing gain—with use of headborne hearing aids occasionally—resulted in social hearing rehabilitation including adequate linguistic progress (Fig. 3). There are apparently certain hearing psychologic factors that constitute an adequate sound stimulation needed for linguistic learning.

Cochlea-complications (dead cochlea) appeared in 4 cases in 3 patients in immediate connection with the operation. One patient primarily had a good hearing improvement, though not a closed air-bone gap but at the 3 month control the preoperative gap was again established. A revision was proposed but the patient did not return until the 12 month control and showed then complete deafness on the operated ear. Tympanotomy revealed that the oval window was completely filled with the vein graft. Normal looking middle ear mucosa covered the tube, which remained firmly in normal position on incus, which only had a very slight groove at the circumference of application but without any sign of necrosis. Thus there were no macroscopic findings that could explain the cochlear lesion. The post

operative cochlear lesions following stapes surgery vary in different case materials according to Beickert's exposé (Beickert, 1963) in Berendes-Link-Zöllner's Handbuch, which are based on ca. 1000 references, there is an average cochlear lesion rate of 4%.

The postoperative impairment of B.C.-level, especially in 2000 and 4000 Hz has often been discussed. In 139 consecutive cases this factor was controlled 12 months after operation, which at 2000 Hz revealed improved H.C.-level (between 5-30 dB) in 108 cases, status quo in 23 cases and a slight impairment (5-15 dB) in 8 cases. The corresponding figures at 4000 Hz were improvement 37 status quo 50 impairment 23 (5-10 dB) and 4 (15-30 dB). The B.C.-levels were also controlled in 61 consecutive cases 2 years after operation. At the 2000 Hz-frequency 47 revealed improvement (between 5-30 dB) 11 status quo and 3 had impaired levels (10-15 dB). At the 4000 Hz frequency improvement in 21 cases status quo in 19 a slight deterioration (5-10 dB) in 15 and in 6 cases an impairment between 15-25 dB. The 6 cases revealing 15-25 dB impairment at 4000 Hz 2 years after operation and the corresponding 4 cases from the one year control were patients of varying age (born between 1891-1932) and had principally all first A.C.-curve types. The age factor seems difficult to evaluate, but worth mentioning is that among these patients there is nobody with a gradual down curve type which statistically should be expected. Therefore there is reason to check postoperative H.C.-levels over a sequence of years, which perhaps may reveal some clue as to which factors constitute these partial cochlear deteriorations.

### CONCLUSIONS

The experiences gained in the actual case series have on the whole been very positive from the hearing rehabilitation point of view especially concerning the possibilities to operate and rehabilitate many "border line" cases for instance old patients using bodyborne hearing aids for many years with audiometrically established severe cochlear lesions. It may often be difficult to compare postoperative hearing results from different publications, because of their different characters from so many aspects (the constitution of A-B-C- and D-cases etc.) A more adequate comparison can be made between some different case materials when a group of cooperating clinics are using the same definitions and evaluations with registration on the same A.D.P.-audiology journals. A team in the Swedish Otolaryng. Society is running such a project.

### ZUSAMMENFASSUNG

An der O.-H. Klinik des Södersjukhuset in Stockholm ist in dem Zeitraum von 1962-1967 an einem Material von insgesamt etwa 500 Otosklerose-Patienten neben anderen Operationsmethoden wahlweise die Stapedektomie, d. h. Abdeckung des ovalen Fensters mit Vena und Implantation eines Polyäthylenschlauches als

Stapes-Ersatz, durchgeführt worden. Wie das aktuelle Material von 309 staphektomierten Patienten erkennen lässt, ist das Alter des Patienten nicht von wesentlicher Bedeutung. Die postoperativen Hörgewinne, die aus dem Ausgleich des Schalleitungsdefizits und der durchschnittlichen Schwellenwertverbesserung im mittleren Frequenzbereich zu entnehmen sind, sind in guter Übereinstimmung mit den Erfahrungen von vielen anderen Autoren. In dem vorliegenden Material ist besonders hervorzuheben, dass viele Patienten, die auf Grund des audiometrischen Befundes als C- bzw. D-Fälle einzustufen sind, gute Operationsergebnisse gezeigt haben und damit scheinen auch für diese Gruppen die prognostischen Aussichten recht gut zu sein. Postoperative Innenohrerraudungen traten nur bei 4 Patienten von insgesamt 300 ein.

Mit Hinblick speziell auf die Rehabilitierung der staphektomierten Patienten war das Resultat im ganzen gesehen sehr günstig. 90% der Patienten konnten entweder ihr Hörgerät gänzlich ablegen oder aber an Stelle des bisherigen Taschengerätes zu einem Hinter-dem-Ohr-Gerät übergehen. Dies ist zweifelsohne ein sehr wesentlicher Gesichtspunkt in der Indikation zu einer Operation.

Ein wesentliches Erfordernis sind kontinuierliche postoperative Hörkontrollen speziell zur Feststellung von eventuell später eintretenden Innenohrerraudungen.

Abschliessend wird vorgeschlagen, bei der Präsentation der verschiedenen Materialzusammenstellungen von einheitlichen Definitionen und Klassifizierungen auszugehen z. B. durch allgemeinen Gebrauch der A.D.P. Journale, wodurch sich wahrscheinlich eine bessere Ausgangsbasis für die prognostische Beurteilung erreichen liesse, was in der Folge eventuell zu einer milder radikalen Operationstechnik führen kann.

## REFERENCES

- ANDERSSON, H. C., and WARREN, H., 1968. Otosclerosis surgery on patients above the age of 64 years. *Geriatric audiology*. Almqvist & Wiksell, Stockholm.
- B. CARRUT, P., 1965. In *Hals-, Nasen-, Ohrenheilkunde* (ed. J. Berendes, R. Link, and F. Zöllner), 13, part 1. Georg Thieme, Stuttgart.
- GOODHILL, V., 1961. *Stapes surgery of deafness*. Huber, New York.
- JOHANSSON, H., 1967. Result of 200 otosclerosis operations. *Scandinavian Journal of Otolaryngology* (Stockh.) Suppl. 224: 115.
- LUNDBERG, T., 1965. Försök till databehandling i audiologisk journal. *Idé och J* 130.
- PORTMAN, M., 1965. La chirurgie stapédienne (Proc. VIII Internat. Cong. O.T.L.). Int. Congress Series n. 112. Excerpta Medica Foundation.
- SERULLA, L., 1965. A safe self locking stapedectomy prosthesis. *Arch. Otolaryng.* (Chic.) 82, 81.
- SNOOK, T., 1953. Otosclerosis kirurgi. *Idé och J* 49: 353.

ENT Department  
Södersjukhuset  
Stockholm, Sweden

Received June 10, 1968

## ATYPICAL CILIA IN NORMAL HUMAN AND GUINEA PIG MIDDLE EAR MUCOSA

I. KAWABATA and M. PAPARELLA

*From the Department of Otolaryngology College of Medical Sciences, University  
of Minnesota, Minneapolis, Minn., U.S.A.*

Normal middle ear mucosa from human and guinea pig material was prepared for electron microscopic studies. Unusual cilia which contain two or more axial filaments complexes were occasionally encountered in these preparations. Detailed observations on the fine structure of these cilia has been presented, discussed and compared with ordinary cilia.

The mucosa of the middle ear and the eustachian tube is continuous with the mucous membrane of the nasopharynx. The lining of the middle ear cavity is also an extension and modification of respiratory mucosa. Cells with cilia in the middle ear of human are morphologically identical to those of the respiratory system (Kawabata & Paparella, in preparation).

In examining the ultrastructure of the normal middle ear mucosa of human and guinea pig, we observed cilia with unusual internal structures. This paper describes these cilia in detail. Several observations concerning unusual cilia in pathological conditions have been described (Duncan & Ramsey 1965 Gallo *et al* 1965).

### MATERIAL AND METHODS

A specimen of middle ear mucosa was surgically obtained from a patient with conductive deafness resulting from head trauma. This patient had no history of inflammatory ear disease. This specimen was fixed with 1% osmium tetroxide in veronal buffer pH. 7.4. The middle ear mucosa from an adult male guinea pig was fixed with 3.5% glutaraldehyde in phosphate buffer pH. 7.4 and the specimen was postfixated in 1% osmium tetroxide and then dehydrated and embedded in Epon 812. Ultrathin sections were cut with glass knives on a LKB microtome, double stained with uranyl acetate and lead citrate, and observed in an RCA EMU-3G electron microscope.

### RESULTS AND DISCUSSION

In recent years electron microscopists have reviewed in detail the complex internal structure of cilia and flagella (Fawcett, 1954 Lansing & Lamy

This work was supported by Public Health Service Research Grant from the National Institutes of Neurological Diseases and Blindness (KB 05970).

1961 Rhodin 1950 Rhodin & Dalhamn 1956 Roth & Shigenaka, 1964) All cilia contain a constant number of eleven internal fibrils, two in the center and nine evenly spaced around them. This pattern is amazingly constant throughout the plant and animal kingdoms (Fawcett, 1961). Rhodin (1966) described the ultrastructure of ciliated cells of human tracheal epithelium. No obvious differences were found between the general morphology of cilia in human middle ear mucosa and the tracheal epithelium.

However cilia containing an unusual arrangement of internal structure were occasionally encountered in both human and guinea pig middle ear mucosa. The internal structure of the cilium is termed by Fawcett (1961) the Axial Filament Complex. It consists of the two central fibers and the nine peripheral fibers mentioned above. In cross section the central fibers are circular and separated from each other. They have a dense surface layer with a less dense interior which gives them a tubular appearance. The outer fibers in cross section are oblong with the short axis running radially and the long axis is divided into halves by a radially oriented septum, which is continuous with the dense surface layer of the fiber and is of the same thickness (Fig. 4). The peripheral fibers have a "double-barreled" or figure eight (Fawcett 1961) appearance in cross section, whereas the central single fibers have a simple circular pattern. This principal pattern, axial filament complex, is usually enveloped by a ciliary membrane which is continuous with the free surface of the ciliated cell. The cilia illustrated in this paper have anomalous internal structures with two or more axial filament complexes in a common ciliary membrane. Its fine structure however appears normal.

FIG. 1. Electron micrograph of guinea pig middle ear mucosa showing profile of an entire usual cilium (arrow). One of the axial filament complexes is directly connected with the basal body (B) but the other is not found in the photograph, presumably due to sectioning directly in the central cilium. Mitochondria. 23,000.

FIG. 2. Electron micrograph of middle ear mucosa of normal guinea pig showing the cross section of cilia. Besides the normal cilia (C) the unusual cilium (arrow) which contains two pairs of axial filament complexes and is enveloped in the normal ciliary membrane can be seen. The entire appearance of this cilium is visible in the inset. 23,000.

FIG. 3. Electron micrograph of normal middle ear mucosa of human showing cilia in cross section. There are two kinds of unusual cilia, one (right arrow) is the same as that seen in Fig. 2, the other (double arrow) has three pairs of axial filament complexes. The shape of the latter appears triangular. Central cilium. 20,300.

FIG. 4. Electron micrograph of an unusual cilium (arrow) of guinea pig middle ear mucosa. In the cross section of this cilium which is covered by a glialillary membrane there are three pairs of central fibers, however the circumferential peripheral fibers correspond to the central ones are not directly represented. The orientation of each central pair is parallel, and this direction is, furthermore parallel to that of the surrounding normal cilium. 23,000.





1961 Rhodin, 1959 Rhodin & Dalhamn 1956 Roth & Shigenaka, 1964) All cilia contain a constant number of eleven internal fibrils, two in the center and nine evenly spaced around them. This pattern is amazingly constant throughout the plant and animal kingdoms (Fawcett, 1961) Rhodin (1966) described the ultrastructure of ciliated cells of human tracheal epithelium. No obvious differences were found between the general morphology of cilia in human middle ear mucosa and the tracheal epithelium.

However cilia containing an unusual arrangement of internal structure were occasionally encountered in both human and guinea pig middle ear mucosa. The internal structure of the cilium is termed by Fawcett (1961) the Axial Filament Complex. It consists of the two central fibers and the nine peripheral fibers mentioned above. In cross section the central fibers are circular and separated from each other. They have a dense surface layer with a less dense interior which gives them a tubular appearance. The outer fibers in cross section are oblong with the short axis running radially and the long axis is divided into halves by a radially oriented septum, which is continuous with the dense surface layer of the fiber and is of the same thickness (Fig. 4). The peripheral fibers have a "double-barreled" or "figure eight" (Fawcett 1961) appearance in cross section, whereas the central single fibers have a simple circular pattern. This principal pattern, axial filament complex, is usually enveloped by a ciliary membrane which is continuous with the free surface of the ciliated cell. The cilia illustrated in this paper have anomalous internal structures with two or more axial filament complexes in a common ciliary membrane. Its fine structure however appears normal.

FIG. 1. Electron micrograph of guinea pig middle ear mucosa showing profile of one entire unusual cilium (arrow). One of the axial filament complexes is directly connected with the basal body (B) but the other is not found in the photograph, presumably due to sectioning direction. C = cilium, M = mitochondrion. 28,000

FIG. 2. Electron micrograph of middle ear mucosa of normal guinea pig showing the cross section of cilia. Besides the normal cilia (C) the unusual cilium (arrow) which contains two pairs of axial filament complexes and is enveloped in the normal ciliary membrane can be seen. The entire appearance of this cilium is club shape. 25,000

FIG. 3. Electron micrograph of normal middle ear mucosa of human showing cilia in cross section. There are two kinds of unusual cilia, one (labeled arrow) is the same as that seen in Fig. 2, the other (labeled arrow) contains three pairs of axial filament complexes. The shape of the latter appears triangular. C = normal cilia. 20,500

FIG. 4. Electron micrograph of an unusual cilium (arrow) of guinea pig middle ear mucosa. In the cross section of this cilium which is covered by a ciliary membrane there are three pairs of central fibers, however the circumference of peripheral fibers corresponding to the central ones are not entirely represented. The orientation of each central pair is parallel, and this direction is, furthermore parallel to that of surrounding normal cilia. 23,000

## REFERENCES

- DE LAY, J. R., and HAMANN, F. K., 1963 Fine structural changes of the porcine nasal ciliated epithelial cell produced by Bordetella Bronchiseptica Rhinitis. *Am. J. Pathol.* 47, 641.
- DE LAY, A. J. III, FLOCK, A., and WESSALL, J., 1966 The ultrastructure of the sensory hairs and associated organelles of the cochlear inner hair cell, with reference to directional sensitivity. *J. Cell Biol.* 29, 497.
- FLETCHER, D. W., 1954 The study of epithelial cilia and peron flagellum with electron microscope. *Laryngoscope* 64, 557.
- 1961 Cilia and flagella. In *The cell* (ed. J. Brachet and A. E. Mirsky) Academic Press, New York.
- FLETCHER, D. W., and PORTER, K. R., 1954 A study of the fine structure of ciliated epithelia. *J. Morphol.* 93, 321.
- GARTO, R. A., G. LOAN, W. H., and HILDRETH, H. A., 1963 Ultrastructure of human nasal papilloma. *Laryngoscope* 73, 144.
- GROSSER, J. R., 1961 The relationship between the fine structure and direction of beat of cilia of lamellibranch molluscs. *J. Biophys. Biochem. Cytol.* 11, 179.
- GROSSER, J. R., and GRANTON, A. V., 1960 On flagellar structure of certain flagellates. *J. Biophys. Biochem. Cytol.* 7, 897.
- LIVING, A. L., and LAMB, P., 1961 Fine structure of the cilia of rotiferans. *J. Biophys. Biochem. Cytol.* 9, 799.
- RUOBY, J., 1959 Ultrastructure of the tracheal ciliated mucosa in rat and man. *Ann. Otol.* 68, 941.
- 1966 Ultrastructure and function of the human tracheal mucosa. *Ann. Rev. Resp. Dis.* 32, 1.
- RUOBY, J. and DALMAN, T., 1958 Electron microscopy of the tracheal ciliated mucosa in rat. *Z. Zellforsch.* 51, 343.
- ROTH, L. E., and SUGIMOTO, Y., 1964 The structure and function of cilia and flagella of various protozoa. *J. Cell Biol.* 20, 249.

M. Paparella, M.D.

Dept. of Otolaryngology University of Minnesota,  
Minneapolis, Minn. 55455, U.S.A.

Received June 10 1968

Some cilia are doubled having two axial filament complexes enveloped by a completely continuous membrane (Figs 2 and 3). Compared with typical cilia around it, the axial filament complex, fine structure shape and size are identical. The appearance of this cilium is oval or oblong in shape. Infrequently a cilium has three axial filament complexes whose fine structure appears normal. The shape of this cilium is triangular compared with the oval shape of the double cilia (Fig. 3).

Furthermore multicilia which have three pairs of central fibers are infrequently seen, but they are not always completely surrounded by paired peripheral fibers (Fig. 4). That this phenomenon is due to artifact remains a possibility. However this does not seem likely because the axial filament complexes are enveloped within a continuous ciliary membrane and the fine structure seems to be well preserved.

Fig. 1 demonstrates the profile of a double cilium. The right and middle cilia in the photograph (Fig. 1 C) have an ordinary profile of axial filament complex, but the left cilium has two pairs of complexes within a single ciliary membrane. The axial filament complex terminates at its base in a basal body at the level of the cell surface (Fawcett 1961, Duvall *et al.* 1966, Gaito *et al.* 1965, Gibbons, 1961, Rhodin & Dalhamn 1956). In the double cilium one basal body continuous with the complex is demonstrated. The second basal body of the complex does not appear in the photograph presumably due to the angle of sectioning.

Coordinated movement of cilia is not well understood at the present time. However several observations suggested a method of discerning the direction of the effective stroke of the cilium (Fawcett & Porter 1954, Duvall *et al.* 1966). The orientation of the central paired filaments is the same in all of the cross sections. Lines drawn through their central axis are parallel, and the direction of the ciliary beat is perpendicular to these lines according to Fawcett & Porter (1954). The orientation of the central filaments in the unusual cilia in our material is parallel to the central filament of typical cilia appearing around them. This arrangement is of interest, but the functional significance if any of the unusual cilia is unknown.

# ACKNOWLEDGMENT

The authors thank Miss Carolyn Sutherland for technical assistance.

# ZUSAMMENFASSUNG

Die normale Mittelohrschleimhaut in Menschen und in Meerschweinchen wurden mit dem Elektronenmikroskop untersucht. Ungewöhnliche Zilien, die zwei oder mehrere axial filament complexes enthalten wurden in diesen Präparaten gefunden. Die ultrastrukturellen, morphologischen Einzelheiten dieser ungewöhnlichen Zilien wurden mit den normalen Zilien verglichen und diskutiert.

## REFERENCES

- DE CAR, J. R., and RANNEY, F. R., 1963 Fine structural changes in the precine nasal ciliated epithelial cell produced by Bordetella Bronchiseptica Rhinitis. *Am. J. Pathol.* 47, 601.
- DE LU, A. J. III, FLOCK, A., and WENZEL, J., 1966 The ultrastructure of the sensory hairs and associated organelles of the cochlear inner hair cell, with reference to directional sensitivity. *J. Cell Biol.* 29, 487.
- FALCETT, D. W., 1954 The study of epithelial cilia and sperm flagella with lectro microscope. *Laryngoscope* 64, 537.
- 1961 Cilia and flagella. In *The cell* (ed. J. Brachet and A. E. Mirsky) Academic Press, New York.
- FALCETT, D. W., and PORTER, E. R., 1964 A study of the fine structure of ciliated epithelia. *J. Morphol.* 113, 221.
- GATTO, R. A., GALLAGHER, W. H. and HOLMAG, D. A., 1965 Ultrastructure of human nasal papilloma. *Laryngoscope* 75, 144.
- GIBSON, J. R., 1961 The relationship between the fine structure and directed movement in gill of lamellibranch mollusc. *J. Biophys. Biochem. Cytol.* 11, 179.
- GIBSON, J. R. and GIBSON, A. V., 1960 Gill flagellar structure in certain flagellates. *J. Biophys. Biochem. Cytol.* 7, 697.
- LIVING, A. I. and LIVING, P., 1961 Fine structure of the cilia of rot rotifers. *J. Biophys. Biochem. Cytol.* 9, 799.
- ROBERT, J., 1959 Ultrastructure of the tracheal ciliated mucosa of rat and man. *Ann. Otol.* 68, 964.
- 1966 Ultrastructure and function of the human tracheal mucosa. *Ann. Rev. Resp. Dis.* 32, 1.
- ROBERT, J. and DALMAN, T., 1958 Electron microscopy of the tracheal ciliated mucosa in rat. *Z. Zellforsch.* 51, 345.
- ROTH, L. E., and SUMMERS, V., 1964 The structure and function of cilia and flagella in various protozoa. *J. Cell Biol.* 20, 219.

M. Pappas, M.D.

Dept. of Otolaryngology University of Minnesota,  
Minneapolis, Minn. 55455, U.S.A.

Received June 10, 1968

## OBLITERATION OF THE CAVITY IN MASTOIDECTOMY

M Tos

*From the E.N.T. Department Glostrup Hospital Copenhagen Denmark*

A modification of Rambo's temporal-musculoplasty for obliterating the cavity following mastoidectomy is described and the early and late operative results in 110 cases of active chronic otitis are presented. The use of retro-auricular tissue together with the temporal muscle gives a long, cranially pedicled flap one end of which is placed into the antrum and the other towards the tip of the mastoid process. To avoid stenosis, the skin of the auditory canal and of the external meatus is incised longitudinally. By this method more than 90% of the ears dried, and there were only a few postoperative complications. The late results showed that the ears remained dry and that there was no recurrence of cholesteatoma or of infection. The results in respect to hearing are briefly mentioned.

Even in the case of a perfectly performed mastoidectomy the patient as well as the surgeon often has great difficulty in keeping the radical cavity dry. Foul smelling detritus or crusts may accumulate there, and in some cases the cavity has to be cleansed at intervals.

For many years, therefore, otologic surgeons have tried to diminish or obliterate the cavity by means of free or pedicled grafts. Free grafts have been of bone (Schiller & Singer 1960) cartilage (Guilford, 1960) or muscle and fascia (Heermann 1962).

Pedicled grafts are made of the tissue surrounding the cavity: temporal muscle based superiorly (Rambo, 1958) or posteriorly (Thomas, 1963) the retro-auricular subcutaneous tissue together with the neck muscles (splenius capitis, sternocleidomastoid) based either superiorly or inferiorly (Guilford, 1961; Elbrønd, 1963).

Palva (1963) used the retro-auricular subcutaneous tissue with perosteum and the retro-auricular muscle for a pedicled graft with a broad base ventrally. Since the flap is usually too small Palva inserted gelfoam between the flap and the bone. Instead of inserting gelfoam Svane-knudsen (1967) preferred leaving a small cavity at the site of the antrum and attic.

Thorburn (1963) used the bipedicled temporal muscle: one pedicle supplying fascia to cover the middle ear and the other one to obliterate the cavity.

The introduction of pedicle grafts for obliterating the cavity is definitely one of the great advances in otologic surgery. It fulfills several demands at the same time: (1) Obliteration or diminution of the cavity, reducing

the retention of detritus to a minimum (2) creation of an empty vascularized layer of tissue in the cavity which will thereby be more easily and more rapidly epithelialized (3) restoration of approximately the same anatomical status in the auditory canal as prior to the operation. This is of particular importance when tympanoplasty is done in the same stage as mastoidectomy but also when tympanoplasty is planned at a later stage.

However the pedicled grafts may often be too small and too short to fill the cavity completely. For instance, the caudally pedicled sternocleidomastoid muscle can hardly reach the antrum and the cranially pedicled temporal muscle can hardly fill the tip of the mastoid process. The object of the present study was to demonstrate the results of using a large, long flap composed of the retro-auricular subcutaneous tissue plus the temporal muscle.

#### MATERIAL AND METHODS

The material comprises 110 ears with active, chronic otitis. In 36 cases the suppuration had lasted 1-3 years, in 74 cases for more than five years. The perforation of the drum was central in two cases and peripheral in all the others. In 31 cases the defect was in the pars flaccida, in 79 cases in the pars tensa, including 29 very large or total perforations. At operation 83 patients were found to have cholesteatoma, 27 granulating processes in the antrum, mastoid process, or middle ear. In 17 instances the ossicular chains were intact, in the others interrupted.

Our method of obliterating the cavity is a modification of Rambo's

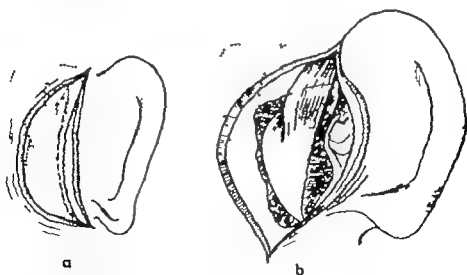


FIG. 1 (a) Incision of the retro-auricular tissue and temporal muscle; (b) the flap of subcutaneous tissue is placed into the cavity so that one part fills the antrum and the other part the tip of the mastoid process.

## OBLITERATION OF THE CAVITY IN MASTOIDECTOMY

M Tos

*From the E.A.T. Department Glostrup Hospital Copenhagen, Denmark*

A modification of Rambo's temporal-musculoplasty for obliterating the cavity following mastoidectomy is described and the early and late operative results in 110 cases of active, chronic otitis are presented. The use of retro-auricular tissue together with the temporal muscle gives a long cranially pedicled flap one end of which is placed into the antrum and the other towards the tip of the mastoid process. To avoid stenosis, the skin of the auditory canal and of the external meatus is incised longitudinally. By this method more than 90% of the ears dried, and there were only a few postoperative complications. The late results showed that the ears remained dry and that there was no recurrence of cholesteatoma or of infection. The results in respect to hearing are briefly mentioned.

Even in the case of a perfectly performed mastoidectomy the patient as well as the surgeon often has great difficulty in keeping the radical cavity dry. Foul smelling detritus or crusts may accumulate there, and in some cases the cavity has to be cleansed at intervals.

For many years therefore, otologic surgeons have tried to diminish or obliterate the cavity by means of free or pedicled grafts. Free grafts have been of bone (Schiller & Singer 1960) cartilage (Gullford, 1960) or muscle and fascia (Heermann 1962).

Pedicled grafts are made of the tissue surrounding the cavity: temporal muscle based superiorly (Rambo 1958) or posteriorly (Thomas, 1963) the retro-auricular subcutaneous tissue together with the neck muscles (splenius capitis, sternocleidomastoid) based either superiorly or inferiorly (Gullford, 1961; Elbrønd 1963).

Palva (1963) used the retro-auricular subcutaneous tissue with periosteum and the retro-auricular muscle for a pedicled graft with a broad base ventrally. Since the flap is usually too small Palva inserted gelfoam between the flap and the bone. Instead of inserting gelfoam Svane-Knudsen (1967) preferred leaving a small cavity at the site of the antrum and attic.

Thorburn (1963) used the bipedicled temporal muscle: one pedicle supplying fascia to cover the middle ear and the other one to obliterate the cavity.

The introduction of pedicle grafts for obliterating the cavity is definitely one of the great advances in otologic surgery. It fulfills several demands at the same time: (1) Obliteration or diminution of the cavity, reducing



## RESULTS

Some 72 ears had dried 3-4 weeks after the operation, 18 ears 6-10 weeks, 9 ears 11-20 weeks, and 13 ears more than 20 weeks after the operation, while in three ears the suppuration never stopped. In 80 cases the cavity had been completely obliterated and presented only as a widened auditory canal. In 54 cases there was a small cavity while in six cases there was a large cavity indicating that the greater part of the flap had undergone necrosis.

In order to avoid retention of the cavity and to get a good view of the drum, we have laid great stress on widening the auditory canal. Three months after the operation the auditory canal and the external auditory meatus were widened in 86 cases. In 13 cases the auditory canal and the meatus were of the same size as prior to the operation. In eight cases the external meatus was rather narrow but yet the cavity and drum could be viewed. In two cases the external meatus was very narrow and this gave rise to marked retention of cerumen.

Postoperative complications occurred in five cases. Two patients developed, a few days after the operation, an abscess in the scar, one a haematoma at the donor site in the temporal region, and two had oedema of the temporal region. The abscesses were incised, the haematoma punctured, and the oedema subsided spontaneously. There have been no other complaints from the donor site, in particular no pain or disturbances of mastication.

To establish the results at long sight, and to ascertain whether cholesteatoma or infection beneath the subcutis-muscle flap would recur because of the obliteration of the cavity the patients were recently seen in a follow-up examination. Out of the 110 operated ears only 103 could be examined. The average follow-up period was about 2 1/2 years. In 69 cases it was one half to two and in 44 cases from 2-8 years.

The late results in the 103 cases showed that 82 ears had been dry ever since the operation, and the patients have not had any aural complaints. In 11 cases there had been intermittent aural discharge during the first 6-12 months after the operation, but thereafter it had ceased. The ears were found to be dry and the cavities were epithelialized at follow up. The average follow up period in these 11 cases was 31 months. In five cases there had been intermittent, and in one case constant, aural discharge, and at follow up there was suppuration in the ear. It came from the middle ear as the cavities were dry and epithelialized. Three patients reported that they had not had aural discharge but at follow up suppuration was present in the cavity while the drum was intact. Only in one case did discharge start more than one year after the operation after having been absent for a whole year.

The width of the auditory canal and the size of the cavity was the same

TABLE 1 *Postoperative air-conduction averages in frequencies 125-2000 cps.*

	Number of cases in various forms of tympanoplasty				
	I	II	III	IV	Total
Normal hearing (0-20 dB)	7	9	3	0	19
Good hearing (21-30 dB)	4	6	9	3	22
Hearing improved more than 15 dB	1	4	6	2	13
Hearing improved from 0 to 10 dB	4	3	5	3	15
Unchanged hearing	0	1	8	1	10
Hearing made worse from 0 to 10 dB	0	0	3	0	3
Hearing made worse more than 15 dB	1	0	2	0	3
Total	17	23	36	9	85

temporal musculoplasty The flap of the temporal muscle is usually too short to reach the floor of the antrum or the tip of the mastoid process. The use of the retro-auricular tissue combined with the temporal muscle gives a long cranially pedicled flap having two parts. One part is placed into the antrum, the others towards the tip of the mastoid process (Fig. 1)

In the great majority of cases we used retro-auricular incision in a few cases Lempert's or Heermann's. The retro-auricular incision was applied about 1½ cm behind the attachment of the auricle. The skin was detached from the subcutaneous tissue which was then incised further anteriorly (Fig. 1a) so that later the subcutis was applicable as a pedicled graft. 85 patients had modified radical operation with tympanoplasty and closure of the perforated drum with fascia. Thirteen had modified radical operation closing the tympanic defect but without reconstruction of the defective ossicular chain. The remaining 12 cases had the conventional radical operation. To avoid later narrowing of the auditory canal the mastoidectomy and tympanoplasty were followed by dilatation of the auditory canal which was incised at 10 o'clock through its entire length. The incision was carried right through the external auditory meatus which was thus also dilated. The auditory canal was packed with gelfoam and hydrocortisone terramycin meche.

The named flap of subcutaneous tissue plus muscle was obtained by an incision as shown in Fig. 1 and, together with periosteum, detached from the skull. With scissors it was then cut so as to make two parts on one pedicle. One part was placed into the antrum and the other part into the mastoid process (Fig. 1b). The fascia covering the tympanic defect also covered the bottom part of the muscle flap.

Postoperatively antibiotics were administered, as a rule penicillin. The meche was changed for the first time on the 10th day thereafter every 3rd day until the ear was dry.

## RESULTS

Some 72 ears had dried 3-5 weeks after the operation, 16 ears 6-10 weeks, 9 ears 11-20 weeks, and 10 ears more than 20 weeks after the operation, while in three ears the suppuration never stopped. In 50 cases the cavity had been completely obliterated and presented only as a widened auditory canal. In 54 cases there was a small cavity while in six cases there was a large cavity indicating that the greater part of the flap had undergone necrosis.

In order to avoid retention of the cavity and to get a good view of the drum, we have laid great stress on widening the auditory canal. Three months after the operation the auditory canal and the external auditory meatus were widened in 86 cases. In 13 cases the auditory canal and the meatus were of the same size as prior to the operation. In eight cases the external meatus was rather narrow but yet the cavity and drum could be viewed. In two cases the external meatus was very narrow and this gave rise to marked retention of cerumen.

Postoperative complications occurred in five cases. Two patients developed, a few days after the operation, an abscess in the scar, one a haematoma at the donor site in the temporal region and two had oedema of the temporal region. The abscesses were incised, the haematoma punctured, and the oedema subsided spontaneously. There have been no other complaints from the donor site, in particular no pain or disturbances of mastication.

To establish the results at long sight, and to ascertain whether cholesteatoma or infection beneath the subcutis-muscle flap would recur because of the obliteration of the cavity the patients were recently seen in a follow up examination. Out of the 110 operated ears only 103 could be examined. The average follow up period was about 2  $\frac{1}{2}$  years. In 59 cases it was one half to two and in 44 cases from 2-6 years.

The late results in the 103 cases showed that 82 ears had been dry ever since the operation, and the patients have not had any aural complaints. In 11 cases there had been intermittent aural discharge during the first 6-12 months after the operation, but thereafter it had ceased. The ears were found to be dry and the cavities were epithelialized at follow-up. The average follow up period in these 11 cases was 31 months. In five cases there had been intermittent, and in one case constant, aural discharge, and at follow-up there was suppuration in the ear. It came from the middle ear as the cavities were dry and epithelialized. Three patients reported that they had not had aural discharge, but at follow up suppuration was present in the cavity while the drum was intact. Only in one case did discharge start more than one year after the operation after having been absent for a whole year.

The width of the auditory canal and the size of the cavity was the same

TABLE 1 *Postoperative air-conduction averages in frequencies 125-2000 cps*

	Number of cases in various forms of tympanoplasty				
	I	II	III	IV	Total
Normal hearing (0-20 dB)	7	9	3	0	19
Good hearing (21-30 dB)	4	8	9	3	22
Hearing improved more than 15 dB	1	4	6	2	13
Hearing improved from 0 to 10 dB	4	3	5	3	15
Unchanged hearing	0	1	8	1	10
Hearing made worse from 0 to 10 dB	0	0	3	0	3
Hearing made worse more than 15 dB	1	0	2	0	3
Total	17	23	36	9	85

temporal musculoplasty. The flap of the temporal muscle is usually too short to reach the floor of the antrum or the tip of the mastoid process. The use of the retro-auricular tissue combined with the temporal muscle gives a long cranially pedicled flap having two parts. One part is placed into the antrum the others towards the tip of the mastoid process (Fig 1).

In the great majority of cases we used retro-auricular incision, in a few cases Lempert's or Heermann's. The retro-auricular incision was applied about 1½ cm behind the attachment of the auricle. The skin was detached from the subcutaneous tissue which was then incised further anteriorly (Fig 1 a) so that later the subcutis was applicable as a pedicled graft. 85 patients had modified radical operation with tympanoplasty and closure of the perforated drum with fascia. Thirteen had modified radical operation closing the tympanic defect, but without reconstruction of the defective ossicular chain. The remaining 12 cases had the conventional radical operation. To avoid later narrowing of the auditory canal the mastoidectomy and tympanoplasty were followed by dilatation of the auditory canal which was incised at 10 o'clock through its entire length. The incision was carried right through the external auditory meatus which was thus also dilated. The auditory canal was packed with gelfoam and hydrocortisone-terracycline meche.

The named flap of subcutaneous tissue plus muscle was obtained by an incision as shown in Fig 1 and together with periosteum detached from the skull. With scissors it was then cut so as to make two parts on one pedicle. One part was placed into the antrum and the other part into the mastoid process (Fig 1 b). The fascia covering the tympanic defect also covered the bottom part of the muscle flap.

Postoperatively antibiotics were administered, as a rule penicillin. The meche was changed for the first time on the 10th day thereafter every 3rd day until the ear was dry.

## MORPHOLOGIC CHANGES IN VESTIBULAR HAIR CELLS IN A STRAIN OF THE WALTZING GUINEA PIG

S. EANSTON, P.-G. LUNDQUIST, E. WEDINBERG and J. WERHALL

*From the Department of Otolaryngology Karolinska Sjukhuset and the King  
Gustaf V Research Institute Stockholm, Sweden*

Genetically deaf animals have been known for many years. Some of these animals combine deafness with a waltzing behaviour. In the present work some characteristics of the morphology of the vestibular hair cells from labyrinths of waltzing guinea pigs of various ages were studied with electron microscopical methods. In survey studies with low magnification clear pathological changes were found in labyrinths from adult animals severely degenerated sensory cells and cellular debris pushed out into the endolymphatic space. With high resolution even the sensory cells of the newborn animals were found to be pathologically changed with variation in sensory hair diameter and balloon-shaped protrusions of the sensory cell apical cytoplasm. In the sensory cells of type I a specific rod-shaped cytoplasmic inclusion was regularly present. This inclusion body was composed of an electron dense substance seemingly consisting of fine tubules or fibrils. The synaptic region was normal in the sensory cells as long as the degeneration of the cell was not very advanced. Both afferent and efferent nerve endings were normally present. The morphological changes demonstrable in the vestibular sensory cells already from the birth lead us to believe the waltzing behaviour to be closely related to this peripheral sensory cell damage. The failure of earlier investigators to demonstrate pathological changes in the sensory epithelium is considered due to the limitations of the light microscopical methods used.

The occurrence of genetic deafness in animals has been recognized for more than 60 years, but the study of such animals has become increasingly rewarding in the last few years through the recent investigations on hereditary deafness in man and the modern morphologic and physiologic methods applied in inner ear research.

In some of these animals the deafness is accompanied by waltzing behaviour. As early as 1929 Haas reported on a strain of the waltzing guinea pig, and early morphologic studies of the inner ear of these animals were carried out by Lurie (1930, 1940), Lurie and Dempsey (1930), Cogan (1940).

Four guinea pigs donated to this department by National Institutes of

This work was supported by U.S. Public Health Service grant No. NS 02334-01-04, the Swedish Medical Research Council, and Harald and Grete Jeansson Stiftelse.

at follow up as at conclusion of the treatment. In particular there had not occurred further narrowing of the external meatus.

As mentioned above, type I-IV tympanoplasty was performed in the same stage as mastoidectomy. The average hearing of the frequencies 125-2000 cps two-three months after the operation is shown in Fig. 2. A total of 41 patients (48.2%) obtained good or normal hearing (30 dB or more). Out of the remaining 44 patients, who did not gain more than 30 dB postoperatively, 13 had gained more than 15 dB and 15 0-10 dB.

### ZUSAMMENFASSUNG

Somit ist eine Modifikation der Temporalis-Muskelplastik von Rambo zur Verödung der Mastoidhöhle beschrieben und die sofortige sowie spätere Operationsergebnisse bei 110 aktiven chronischen Mittelohrentzündungen dargestellt. Bei Anwendung der retroaurikulären Gewebe zusammen mit dem Musculus temporalis bekommt man einen langen kranial gestielten Lappen, von welchem man einen Teil in den Antrum und den anderen zur Spitze des Processus mastoideus einführt. Um die Stenose des Gehörganges zu vermeiden, schneidet man die Haut des Gehörganges und des Meatus akustikus externus in der ganzen Länge auf.

Bei dieser Methode wurden die Ohren bei über 90% der Fälle trocken und wir hatten wenige postoperative Komplikationen. Spätresultate zeigten, dass die Ohren trocken blieben und dass es nicht zum Rezidiv des Cholesteatomes oder zu Rezidiventzündungen kam. Die Ergebnisse der Hörverbesserung werden kurzfassend dargestellt.

### REFERENCES

- ELBERG, O. 1963 Obliteration of mastoid cavity with temporal bone surgery. *Arch. Otolaryng.* (Chic.) 78: 132.
- CULLPORN, F. R. 1960 Controlled cavity healing after mastoid and fenestration operations. *Arch. Otolaryng.* (Chic.) 71: 165.
- 1961 Obliteration of the cavity and reconstruction of the auditory canal in temporal bone surgery. *Trans. Amer. Acad. Otolaryng.* 65: 114.
- HEERMAN, J. 1962 Experiences with free grafts of fascial connective tissue of the temporal muscle in tympanoplasty and in filling up the cavity after radical mastoidectomy. A cartilaginous bridge from the stapes to the lower margin of the eardrum. *N. Laryng. Rhin. Otol.* 41: 141.
- PALVA, T. 1963 Middle ear surgery in northern Europe. *Arch. Otolaryng.* (Chic.) 78: 363.
- RAMBO, J. H. T. 1958 Primary closure of the radical mastoidectomy wound. A technique to eliminate postoperative ear. *Laryngoscope* 68: 1216.
- SCHILLER, A. and SINGER, M. 1960 Mastoidectomy with gustomy and cancellous bone. A new procedure. *S. Afr. Med. J.* 35: 645.
- SVANE, H. DR. V. 1967 Mastoidectomy with muscular obliteration modified after Palva. *Acta Otolaryng.* (Stockh.) Suppl. 223: 334.
- THOMAS, H. V. 1963 Chronic middle ear infection—mastoid surgery without mastoidectomy. *J. Laryng.* 77: 63.
- THOMSEN, I. B. 1963 Tympanoplasty. Principles and use of pedicled temporal muscle flaps. *J. Laryng.* 73: 801.

K. T. D. p. m. n. l. G. l. s. t. u. p.  
Hospit. i. Copenhagen u. D. mark.

Received September 4 1968

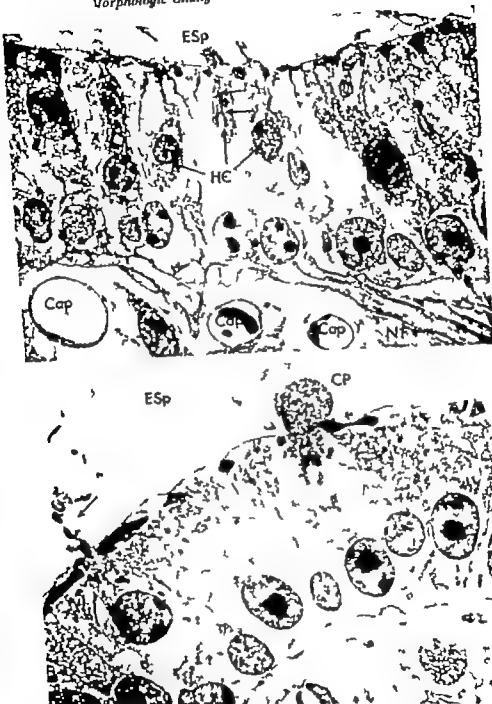


FIG. 1. Electron microscope survey of cristae ampullaris from a 2-day-old (A) and an 11-month-old (B) white guinea pig. Note the decrease in the number of the hair cells (A). Protrusions of the hair cell tips can be found here. A nerve fiber (NF) is penetrating the basement membrane and losing its myelin sheath. In one of the hair cells of type I, inclusion bodies are visible (arrows). Capillaries (Cap) are found in the connective tissue. Esp, Endolymphatic space; HC, Hair cells. (B) Only a few hair cells are still present. The hair cell tips found here protrude into the endolymph with balloon-shaped cytoplasmic projections (CP) and the base of the sensory hairs are clumped together (arrow). (A) 187 $\times$ , 1500; (B) 77 $\times$ , 2 $\times$ 100.

Health in 1961 have since been bred with normal "Swedish" guinea pigs, this providing the opportunity to analyse the genetic characteristics, physiology and morphology of these animals. The present study was undertaken to examine some characteristics of the morphology of the vestibular hair cells from the labyrinths of waltzing guinea pigs of various ages.

## METHODS

### *Light Microscopy*

The waltzing guinea pigs ranged in age from newborn to 2 years old. Fixation was performed as follows. After inducing anaesthesia with Nembutal the thorax was opened a cannula was inserted in the ascending aorta, and perfusion was carried out with isotonic saline followed by the Heldenbain-Susa fixative solution. After perfusion with approximately 500 ml the animal was decapitated, the head immersed in fixative and subsequently embedded by the celloidin technique (Lundquist, 1965). The specimens were orientated with the modiolus of the cochlea in the same plane as, and parallel to the knife. 15  $\mu$  serial sections were cut on a celloidin microtome. These were stained with haematoxylin and eosin and by the Mallory azan method.

For detailed morphologic examination 1  $\mu$  sections were cut from Epon embedded specimens, stained with toluidine blue and examined by light and phase-contrast microscopy.

### *Electron Microscopy*

After anaesthetizing with chloroform and decapitation the temporal bones were dissected and the bullae tympanicae opened. The round window was extirpated, the stapes removed, and the apex of the cochlea opened. The specimens were then perfused with 1 per cent osmium tetroxide solution (Rhodin 1954) dissected under an operating microscope, and the various parts of the labyrinths embedded by the standard Epon procedure (cf Luft 1961 Kay 1965 Sjöstrand 1967).

After sectioning on a LKB Ultratome the specimens were stained with uranyl acetate and lead acetate (Walson, 1958 Karnovsky 1961) and examined with a Siemens Elmiskope I. For photography Gevaert Scientia 23 D 50 plates were used with primary magnifications ranging from 1000 to 40 000.

## RESULTS

### *Light Microscopy*

The celloidin sections showed no significant changes of the morphology of the semicircular canals, ampullae, utricle, and saccule as seen in the light microscope. The ampullae were provided with a normal cupula. The sensory epithelia in the older animals, however, showed a suspected reduc-





tion in the number of sensory cells but the celloidin sections did not allow a satisfactory analysis of the relation between the number of sensory cells and supporting cells.

The same was true for the epithelium on the saccular and utricular maculae.

### *Electron Microscopy*

#### *Epithelial cytoarchitecture*

A general examination of the vestibular sensory epithelia at low magnification disclosed no conspicuous malformations in the newborn animals. There seemed to be a normal relationship between the types I and II sensory cells (Wersäll 1956). In the older animals, however, even the survey pictures showed the presence of definite pathologic changes, with a considerable decrease in the number of both types of sensory cells. Severely degenerated sensory cells and cellular debris were found between the supporting cells and in the endolymphatic space on the surface of the epithelium. A comparison between newborn and older animals indicated an obviously progressive degeneration of the sensory cells. The supporting structures were apparently normal and nerve fibers were identified (Fig. 1).

#### *Sensory hairs*

Even in the newborn animals a slight variation in the hair diameter was found (Fig. 2 A). The diameter of the kinocilia was normal. Examination of their tubular filaments disclosed the absence of central tubules in some cilia and supernumerary tubules in others. Balloon shaped protrusions of the apical cytoplasm were found in the majority of the sensory cells, usually in the periphery of the bundle.

A pronounced distortion of the hair bundle configuration which increased with age was observed (Fig. 2 A-C). In many of the animals the normal morphologic polarization of the sensory cells (Wersäll *et al.* 1964) was severely disturbed. The sensory hairs varied widely in appearance, sometimes with grotesque deformities. In the greatly swollen hairs the plasma membrane was often ruptured. Hair remnants were found in various stages of disintegration.

#### *Hair cell top*

In the newborn animal the density and configuration of the cuticle of the sensory hair cells was apparently normal. The hair rootlets were inserted

FIG. 2. This montage illustrates the progressive degeneration of the sensory hairs. (A) Newborn with varying diameters of the hairs (arrows). Sometimes a normal appearing hair bundle is found (HB). (B) With increasing age (3 days) increasing disconfiguration is found, and the kinocilia (KC) are sometimes pathologically in appearance with supernumerary tubules (KCP). (C) In advanced degeneration (58 days) rich profusion of cytoplasmic debris (CgD) is found. (A) 376 $\times$ , 4500 $\times$ , (B) 330 $\times$ , 14,300 $\times$ , (C) 127 $\times$ , 18,000 $\times$ .



FIG. 3. In cross section the rod-shaped structure is rounded in appearance and can be found close to the nuclei (arrows). The nuclear border at this region is indented. The fact also described is found only in the type I cell (HC I). VCh, Very chalice; SC, secretory cell. Age: 18 months. 248 $\times$  14,600.



FIG. 4. Low power electron micrograph showing hair cell of type I (HC I) with rod-shaped innervation bodies passing close to and indenting the nucleus (arrows). Other hair cells of type Ia with supporting cells with their basally located nucleus (SC Ia) are present. The afferent nerve fibers forming the nerve, helices are seen penetrating through the basement membrane (V/H). Age 80 days. 142V. 6000.



FIG 5. In cross section the rod-shaped structure is rounded in appearance and can be found close to the nuclei (arrows). The nuclear border at this region is indicated. The inclusion described is found only in the type I cell (HC I). VCh, Vestibular secretory cell. Age 18 months. 248 $\times$  14,600.



FIG. 6. (A) The ultrastructure of a secretory cell tip (HCE) where the nucleus (N) is asymmetrical with a tight nucleus border due to the bypass of rod shaped structure (R) close to the cell. NGR Nervous ganglion, Sc secretory cell. (B) The rod shaped structure (R) is found to penetrate deeply into the vacuolar region pushing back the hair cell cytoplasm ahead. NE Nervous ending. Ig: 80 days. (A) 142 $\times$  12,600. (B) 142 $\times$  21,600.



FIG. 1. Hair cell type I, subsynaptic region. At the top of the picture the nucleus (N). Among the normally present cytoplasmic organelles such as mitochondria (MI), lysosome-like structure (Lg), Golgi apparatus (GA), and multivesicular body (MB), fibrillar condensation is found in the cytoplasm (arrow), similar in structure to that shown by the red-shaped inclusion bodies. NCB, Cross-chalice secretory cell (SC) filled with granules (G). Ag 88 days, 127V, 32,808.

### Synaptic region

So long as the degeneration of the cell was not greatly advanced the synaptic region in the sensory cell was normal. Normal synaptic bars and vesicles were regularly observed (Weraill *et al.*, 1967). Synaptic bars were found in the immediate neighbourhood of the rod-shaped inclusions in a few cells.

### Nerve Fibers and Nerve Endings

The efferent and afferent nerve endings in the epithelium of the newborn and the older walking guinea pigs were apparently normal in number. There appeared to be no degeneration of either the nerve fibers or nerve



FIG. 8. Detail of apical part of hair cell, type I. A protrusion extends to the lymphatic space. In the top of a rod-shaped cell body (R) are normally occurring organelles, such as mitochondria (M), centriole (Cl), and lysosome-like structure (Lg). Ag. 8 months 337A 36,200

endings, and apparently normal endings were observed even in the areas where all sensory cells were completely destroyed and/or expelled from the sensory epithelium.



## Supporting Cells

The supporting cells contained a normal amount of secretory granules, mitochondria and Golgi apparatus. There were apparently no changes during or after the degeneration of the sensory cells.

## DISCUSSION

Light microscopic studies of the labyrinth in the walking guinea pig revealed normal vestibular epithelia (Lurie, 1939). This, together with the absence of any vestibular responses to clinical tests have led several investigators to ascribe the walking behaviour to a centrally located lesion (Cogan, 1940). Experimental surgery in the mesencephalon has resulted in a walking-like behaviour (Lurie & Dempsey 1939).

The marked degeneration of the vestibular sensory cells with morphologically normal nerve endings observed in the present study would indicate, however, that the walking behaviour is closely related to this peripheral damage. The fact that the behaviour was found already from birth is consistent with the observation of the progressive peripherally located degenerative changes in the newborn animals. It seems probable that this degeneration starts prenatally. It is still an open question whether the neuroepithelia of the vestibular organ in these animals mature completely before onset of the degeneration.

The intracellular rod shaped inclusions have not been reported hitherto. Their substructure is suggestive of a filamentous or tubular arrangement. This might indicate that the rod is composed of a protein conceivably originating from a defect in protein metabolism. It is interesting to note that the formation of a presumably pathologic protein that is visible in the electron microscope can be initiated in cochlear hair cells by kanamycin intoxication as shown by Weraffil *et al.* (1967).

## ZUSAMMENFASSUNG

Genetisch bedingte Taubheit bei Tieren ist seit vielen Jahren bekannt. Einige dieser Tiere haben neben ihrer Taubheit auch eine eigenartige, wackel-ähnliche Art zu laufen. In dieser Arbeit wurden einige morphologische Besonderheiten der Vestibular-Haar-Zellen im Labyrinth wackelnder Meerschweinchen verschiedener Alters mit elektronenmikroskopischen Methoden untersucht. Bei Übersichtsstudien mit geringer Vergrößerung fand man deutliche pathologische Veränderungen im Labyrinth von erwachsenen Tieren, z.B. stark degenerierte Sinneszellen und Zellschritter, der das Spatium endolymphaticum umgab. Bei starker Vergrößerung fand man sogar die Sinneszellen neugeborener Tiere pathologisch verändert mit Abweichungen im Durchmesser der Sinneshaare und ballonartigen Ausstülpungen des apikalen Zellplasmas. In den Sinneszellen des Typ I fand man regelmäßig einen spezifischen stabförmigen Zelleinschluss. Dieses Einschlusskörperchen schien aus einer elektroendolithen Substanz zu bestehen, dem Anschein nach feinen Tubuli oder Fibrillen. Der Synapsenbereich der Sinneszellen war normal, solange die Zelldegeneration nicht allzu fortgeschritten war. Sowohl afferent als auch efferent Nervenendigungen waren normal. Die schon von der

Geburt erkennbaren morphologischen Veränderungen der Vestibularis-Sinneszellen machen es wahrscheinlich daß die abweichende Art zu laufen auf einen periferen Schaden der Sinneszellen beruht. Daß man früher keine Veränderungen in den Vestibularis-Sinneszellen waltender Meerschweinchen fand beruht auf dem begrenzten Auflösungsvermögen des Lichtmikroskopes.

## REFERENCES

- ANDERSON, H. and WEDENBERG, E., 1968: Audiometric identification of normal hearing carriers of genes for deafness. *Acta Otolaryng.* (Stockh.) 65 6.
- BUCHANAN, A. R., 1940: Circling in guinea pig with lesions in the brain stem. *Proc. Soc. Exp. Biol. Med.* 43 389.
- COGAX, H. G., 1940: Waltzing guinea pigs with particular reference to ocular movements and righting reflex. *Arch. Ophthalm.* 24 8.
- IRSEK, L. H., 1932: Modifying factors in guinea pigs. *Proc. VI Intern. Cong. Genet.* (Liban.) 2, 97.
- IRSEK, L. H. and RISTY, T. H., 1929: A new character in guinea pigs, waltzing. *Anat. Rec.* 44 294.
- KARNOVATY, M. J., 1961: Simple methods for staining with lead at high pH in electron microscopy. *J. Biophys. Biochem. Cytol.* 11 729.
- KAY, H. H., 1965: *Techniques of electron microscopy* (ed. Desm. and Kay) 2nd ed. Blackwell Scientific Publ. Oxford.
- LIPT, J. H., 1961: Improvement in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9 409.
- LUNDQVIST, P.-G., 1965: The endolymphatic duct and sac in the guinea pig—an electron microscopic and perilymph investigation. *Acta Otolaryng.* (Stockh.) 5 ppl 261.
- LURIE, M. H., 1939: Studies of the waltzing guinea pig. *Laryngoscope* 49 339.
- LURIE, M. H., 1940: Studies of the bred and inherited condition in animals. *J. Anat. Soc. Am.* 11 420.
- LURIE, M. H., 1941: The waltzing (circling) guinea pig. *Ann. Otol.* 50 113.
- LURIE, M. H. and DEWESE, E. W., 1939: Experimentally induced circling (waltzing) in the guinea pig. *Laryngoscope* 49 565.
- RHODIN, J., 1954: *Cytophotomikroskopische Untersuchung der ultrastrukturellen Organisation und Funktion in der Niere und experimentell veränderten proximalen convoluted tubulären Zellen der menschlichen Niere*. Thesis, Karolinska Institutet.
- SÖSTRAND, F. S., 1967: *Electron microscopy of cells and tissues*. Vol. 1 Academic Press, New York and London.
- WATSON, M. L., 1968: Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4 727.
- WERNALL, J., 1965: Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig. A light and electron microscopic investigation. *Acta Otolaryng.* (Stockh.) Suppl. 126.
- WERNALL, J. and FLOCK, A., 1964: Physiological aspects on the structure of vestibular endorgans. *Acta Otolaryng.* (Stockh.) 3 ppl 192-85.
- WERNALL, J., FLOCK, A. and LUNDQVIST, P.-G., 1965: Structural basis for directional sensitivity in ocular and vestibular sensory receptors. *Cold Spring Harbor Symp. Quant. Biol.* 30 115.
- WERNALL, J., GLENNER, L. and LUNDQVIST, P.-G., 1967: Ultrastructure of the vestibular end organ. In *Megaklinetik und vestibuläres System* (ed. A. V. S. d. H. L. and J. Knight) p. 105. Ciba Foundation, London.

S. Ernstson, M.D., Dept. of Otolaryngology  
Karolinska Sjukhuset, Stockholm 60  
Sweden

Received May 5 1968

## HORIZONTAL NYSTAGMUS OF RHESUS MONKEYS

A. KOMATSUBAKI, H. E. HARRIS, J. ALPERT and B. COHEN

From the Department of Neurology Mount Sinai School of Medicine New York  
N.Y. U.S.A.

Horizontal optokinetic nystagmus (OKN), caloric nystagmus, and positional alcohol nystagmus (PAN) of the rhesus monkey (*Macaca mulatta*) were surveyed using electrooculography. OKN of the monkey appears to be similar in most respects to that of man although optokinetic after-nystagmus is more prominent in the monkey. Of the parameters of OKN which were measured, slow phase velocity was most closely related to the pickered stimulus. Caloric nystagmus is easily induced in the monkey and parameters of caloric nystagmus declined exponentially on repeated testing. The first phase of positional alcohol nystagmus was present in all animals tested and the secondary phase in some. The data indicate that oculomotor findings in the monkey can probably be applied to man with few reservations.

The monkey is a good subject for oculomotor studies. Both caloric and optokinetic nystagmus can easily be elicited in the monkey (Krieger & Bender 1936; Shanzler & Bender 1959) and its nervous system is anatomically similar to that of man (Arlens, Kappers et al 1960; Salder & Lee, 1961). Recent papers have analyzed saccadic and pursuit movements of the stump-tailed monkey *Macaca speciosa* (Fuchs, 1967) and the threshold for rotatory nystagmus of the rhesus monkey *Macaca mulatta* (Lange, 1967). This paper is a preliminary survey of various types of horizontal nystagmus of the normal rhesus monkey with particular emphasis on optokinetic nystagmus. The purpose is to provide a basis for comparing nystagmus of monkeys before and after lesions of the central nervous system and aid in comparisons of the oculomotor system of monkey and man.

### METHODS

Juvenile monkeys of the species, *Macaca mulatta*, weighing 2.5-3 kg served as experimental subjects. During testing, monkeys sat in a primate chair with arms and legs restrained and head held by a sponge-covered clamp. Animals were examined in light for saccadic movements, spontaneous nystagmus, gaze nystagmus, and optokinetic nystagmus (OKN). They were tested in darkness for spontaneous nystagmus, positional alco-

This work supported by USPHS Research Grant NS-00781, the Abramson Foundation, USPHS Training Grant NS-0072, and USPHS Career Research Development Award 1 K2-NS-31, 887.

hol nystagmus, caloric nystagmus, and optokinetic after nystagmus (OKAN)

The optokinetic stimulus was an internally lighted rotating drum, 30 inches in diameter with 1 inch black stripes at each 45° (Krieger & Bender 1956). The drum filled the monkey's field of vision and rotated at a constant angular velocity. Drum velocity was servo-controlled to maintain a constant rate of rotation. The rate of rotation was calibrated by counting the stripes which passed a photocell. Eight stripes represented 360° of drum rotation in the normal testing situation. In two experiments (Fig. 4C-D) the number of stripes was doubled. Caloric nystagmus was induced by injecting 15 cc of water at 27.5°C and 47.5°C into the external auditory canal for 15 seconds. This is 10 degrees above and below the monkey's body temperature. The head was extended so that the horizontal semicircular canals were vertical. Caloric nystagmus and OKAN were recorded in darkness.

The recording system is demonstrated in Fig. 1. The upper line is the time base at 1 mark/sec. The second trace is a photocell recording of the passage of black stripes. The third trace is a recording of the corneoretinal potential (EOG) with DC-coupling through platinum needle electrodes inserted at the lateral canthi. The EOG amplifiers were chopper stabilized and had a bandpass from DC to 150 cps. For recordings of Figs. 1, 6 and 7 filtering was added to attenuate frequencies above 22 cps. The recordings of Figs. 2 and 3 and those used in determining the parameters of optokinetic nystagmus (Figs. 4 and 5) were taken without these filters.

The fourth trace of Fig. 1 is the differentiated EOG showing momentary angular eye velocity (Henriksson, 1955; 1956). This trace was used to measure the velocity of quick phases of nystagmus. The differentiators had a high frequency bandpass of 150 cps and a time constant of 3 msec. They accurately phase shifted a 1 millivolt 20 cps sine wave introduced through the EOG input by 90°. The maximum velocity of this signal was equal to that of an eye movement of about 1800°/sec. In the recordings of Figs. 1, 6 and 7 filters were added to the differentiators which reduced the maximum velocity of the eye movement signals passed without distortion to about 500°/sec.

The differentiated EOG was also amplified and rectified (fifth trace). Slow phase eye velocity) to determine angular velocity of the slow phases of nystagmus in isolation. By integrating the rectified slow phase eye velocity (sixth trace) total eye deviation was obtained. When the integrator pen reached the limit of its excursion it returned to the baseline and started again. This accounts for the "sawtooth" nature of the integrated trace during nystagmus.

Nystagmus frequency is shown in the seventh trace in a recording of beat to beat frequency. Considerable variability was usually present in beat to beat frequency so that beats of nystagmus were also counted separately for set periods of time. The vertical bar on the right of the EOG trace

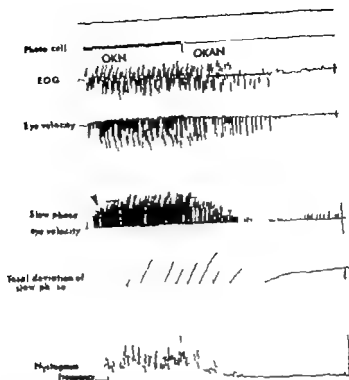


FIG. 1 OKN and OKAN of normal monkey in response to an optokinetic drum rotating at  $90^\circ/\text{sec}$ . The time base is above but at  $1 \text{ ms/k/sec}$ . The passage of each stripe caused deflection in the photocell trace. The sharp downward deflection in the photocell trace also the time when the lights were extinguished. OKN was recorded in dark areas. The initial OKAN was to the left and was followed by small beats of OKAN to the right. The downward arrow in the initial portions of the slow phase eye velocity trace indicates the period during which the velocity of slow phases gradually increased up to about  $90^\circ/\text{sec}$ .

represents about 10° of eye deviation. The EOG was calibrated by alternately shining the monkey lights separated by 15 or 30 in a dimly lit room and averaging a large number of responses. Calibrations are approximate rather than absolute values. Vertical bars on the right of the differentiated traces are approximately 100°/sec and on the right of the integrated trace approximately 100° of deviation. In each of the recordings eye movements to the right caused upward pen deflections.

Parameters of OKN were measured only in animals which were consistently attentive to the optokinetic stimulus when alert. These were about 20 to 40% of the animals tested. A constant state of alertness was maintained by injecting 0.5 mg/kg of amphetamine sulfate intramuscularly at least 30 minutes before testing. Amphetamine does not change the characteristics of nystagmus (Collins & Poe 1963; Cohen *et al* 1960). Two types of optokinetic stimulation were used in this study to induce nystagmus.

1 Drum rotation at a constant velocity of 90 /sec for a period of 45 seconds as shown in Fig 1. At the conclusion of this period the lights were extinguished and optokinetic after nystagmus (OkAN) was recorded in darkness.

2 Drum rotation for periods of 30-45 seconds at constant velocities between 30 -180 /sec. Step increases in drum velocity always progressed from lower to higher velocities for reasons which will be given subsequently.

## RESULTS

The response of a normal monkey to a 45 sec period of constant optokinetic drum rotation at 90 /sec (2 stripes/sec) is shown in Fig 1. The first eye movement was a slow phase to the right followed by quick phases to the left. The eyes beat across the midposition during most of the OKN. The approximate midposition is shown by the dotted line. The amplitude of the beats varied between about 10 and 30°. Quick phase velocities varied but generally did not exceed 400 /sec.

In this as in other animals the eyes did not attain the greatest slow phase eye velocity immediately after the stimulus began. Instead over the first 10 seconds of OkN the maximum velocity of the individual slow phases gradually increased. This period is marked in Fig 1 by the downward arrow over the slow phase eye velocity trace. The frequency during this time was relatively constant. Because of the initial lag in attaining high velocity slow phases, the response to optokinetic stimuli was measured after the first 15 seconds of OKN and the speed of drum rotation was increased from lower to higher velocities.

After the first 10 seconds of OKN the maximum velocity of slow phases varied around 90 /sec. The dotted line through the top of the Slow phase eye velocity trace shows approximately 90 /sec. Integration of slow phase velocity indicates that the eyes moved about 2250° during the 45 sec period of optokinetic stimulation while the drum was travelling 1050°. If the initial 15 seconds of OkN is disregarded, the eyes travelled about 1650° during slow phases in the last 30 seconds of OKN while the drum was travelling 2700°. The mean duration of quick phases was about 50 msec (unpublished data) and the mean frequency was about 4 beats/sec. If 20% is subtracted from 2700° for time spent in quick phases, then 2160° is the arc traversed by the rotating drum during the time the eyes were moving 1650° in the slow phases.

The basis for the difference in the angle of drum rotation and of eye deviation during slow phases of OkN was probably largely due to the fact that the eyes did not move with constant velocity during the slow phases. Occasionally slow phases were of constant velocity particularly at lower OkN drum velocities. More often, however the velocity of slow phases was not constant but fell steadily as the beats progressed (Fig 2, 33 /sec). At higher drum speeds (98 /sec) the velocity of slow phases fell steadily in

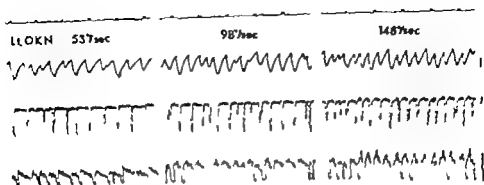


FIG. 2. OKN of normal monkey. The top trace is the time base at 1/sec. The second trace is the EOG, the third trace the differentiated EOG, and the fourth trace the differentiated EOG with the clarity of the quick phases clipped to show slow phase clarity (indicated). The clarity of the OKN drum is marked above each series of traces. Calibrations as in Fig. 1.

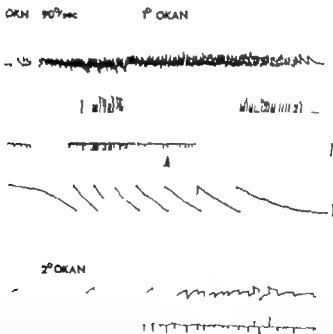


FIG. 3. OKN and OKAN of normal monkey. The lights were extinguished at the downward arrow. The recording scheme is similar to that in Fig. 1 except that only the EOG and differentiated EOG are shown during secondary OKAN (2° OKAN). The recordings are continuous, not the relatively linear decline in slow phase clarity during primary OKAN (primary arrow).

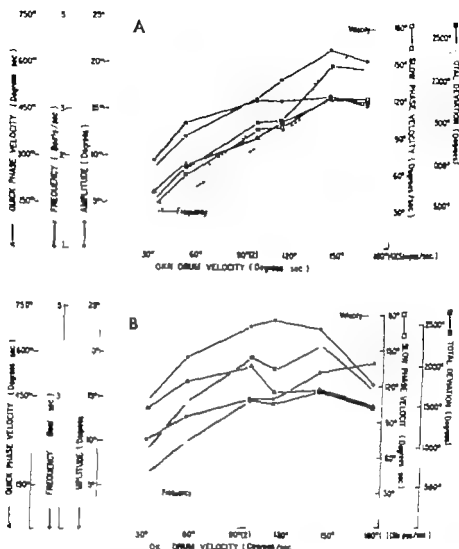
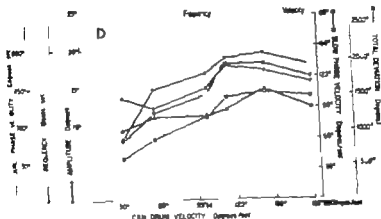
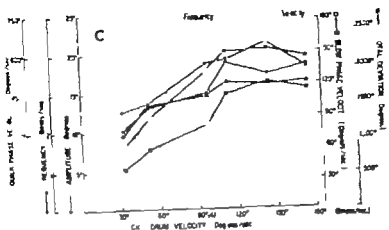


FIG. 4. Graphs of parameters of OKN of the monkey. Graph A and C were obtained from data recorded on the same date and B and D from data taken 5 days later. A and B are the standard test situation with eight stripes on the OKN drum. In C and D the number of stripes was doubled. Each point is the mean value of a single parameter over a 30-sec trial period. The velocity and frequency of the OKN stimulus are shown on the abscissa in degrees/sec and stripes/sec. Perfect following of slow phase velocity is degrees/sec or frequency in beats/sec would follow the dotted lines marked "Velocity" and "Frequency". The closed boxes are symbols for total deviation in degrees, the open boxes for maximum slow phase velocity in degrees/sec, the open triangles for maximum quick phase velocity in degrees/sec, the closed circles for amplitude of beats in degrees, and the open circles for frequency in beats/sec.

some beats while in other it reached a maximum somewhere in the middle of the beat. At still higher drum speeds (148 /sec) eye velocity initially rose and then fell in most beats. With prolonged stimulation at higher drum velocities the velocity configurations often varied, there being a mixture of beats with rising and falling velocities.

Beats of OKN with slow phase velocity which appeared to fall exponentially have been described in the cat (Honrubia *et al.*, 1967) although spe-





elic tests for exponentiality were not performed. Beats with similar configuration also occurred during slow nystagmus in the monkey e.g. during the OhAN of Fig. 1 and the secondary OhAN of Fig. 3. During more frequent nystagmus these beats were much less common than other varieties of slow phases which have been described.

#### *Optokinetic After Nystagmus (OKAN)*

Optokinetic after-nystagmus (OKAN) with quick phases in the same direction as the preceding OhN is prominent in the monkey in darkness (Krieger & Bender 1956 and Figs. 1 and 3). In light OhAN is of short duration or is absent. OhAN was found in every alert monkey which had OKN. OhAN generally lasted for about 60-90 seconds after the end of OKN (Krieger & Bender 1956). The period of OKN necessary to elicit full-duration OhAN varied in different animals. In some monkeys 10-15 seconds of OhN were sufficient to induce OKAN of full duration. Periods of OhN longer than 30 seconds did not lengthen the period of OhAN. Postrotatory nystagmus is not similarly prolonged in darkness in the monkey.

During OKAN the amplitude of the beats was relatively constant but both the frequency and maximum slow phase velocity slowly declined. Usually but not invariably the decline in maximum slow phase velocity during OKAN was linear (Fig 3 upward arrow slow phase velocity trace). The maximum slow phase velocity of the beats at the onset of OKAN was usually somewhat lower than the velocity of the slow phases of OKN which had just preceded them (Fig 3). Primary OKAN i.e. that with quick phases in the same direction as the preceding OKN was followed by secondary OKAN with quick phases in the opposite direction in some animals (Figs. 1 and 3). Secondary OKAN also occurs in humans (Morimoto *et al* 1983). In the animals whose records are shown in Figs. 1 and 3 oppositely directed primary and secondary OKAN were induced when the direction of the stimulus was reversed.

### *Parameters of OKN*

Since a randomly selected group of monkeys was not studied, no attempt was made to statistically define the normal range of OKN in the monkey. Instead the response of several apparently normal monkeys with very good OKN was studied. The relationship of parameters of OKN of one of these monkeys to six different speeds of drum rotation between 30 and 180 /sec is graphed in Figs 4 and 5. Samples of OKN from this animal are shown in Fig 2.

Despite some variability in each of the parameters of OKN recorded in the same animal on different days (Fig 4 4 B) within limits as the velocity of the OKN drum increased, each parameter of nystagmus also tended to increase (Fig 5). The beats of nystagmus became more frequent, the average amplitude of the beats became larger, the maximum quick and slow phase velocities increased and the eyes deviated farther in response to the stimulus.

In this as in other monkeys with active OKN the eyes were able to approximate the velocity of the optokinetic drum during the slow phases of OKN up to drum velocities between 90 and 120 per second. In each of these animals the maximum velocity of some of the beats of OKN exceeded the velocity of the OKN drum at speeds up to 90 /sec. In Fig 4B this tendency was pronounced and the mean of the maximum velocities (open squares) was higher than velocity of drum rotation up to 90 /sec. This was also reflected in a higher total deviation of the eyes at drum velocities up to 90 /sec in this instance (Fig 4B closed squares). OKN following at slow phase velocities up to 90 /sec has also been reported in man (Griffner 1939, Ueda & Suzuki, 1965). Between 120 and 150 /sec the maximum velocity of the slow phases usually increased along with speed of drum rotation, but the eyes did not approximate the velocity of the OKN drum (Fig 5). Above 150 /sec the maximum velocity of the slow phases often fell. For this reason 180 /sec was used as the upper limit of drum velocity for testing.

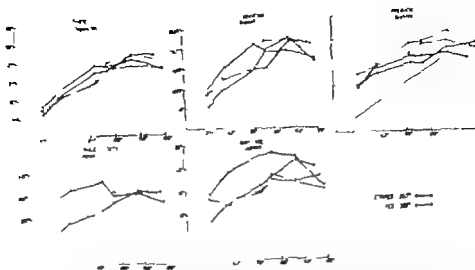


FIG. 5. Data of Fig. 4 replotted for individual parameters. The solid lines in each graph show the response to the normal test situation (8 lines/360°). The dotted lines show the response to doubling the number of stripes in the OKN drum. The underlying dotted line in the graph of slow phase velocity shows perfect following for each velocity of drum rotation. In the graph of frequency the underlying solid line shows frequency changes which would occur if one beat of nystagmus corresponded to the passing of one stripe when there were 8 stripes/360°. The dotted line shows 1:1 following for 16 stripes/360°.

Changes in total deviation (Fig. 4 closed squares, and Fig. 5) paralleled changes in maximum slow phase velocity. That is, the curves tended to be linearly related to the stimulus up to about 90 to 120 /sec. Between 120 and 150 /sec the curves generally increased but not at the same rate as the stimulus. Above 150 /sec total deviation fell. There was more variation in total deviation than in maximum slow phase velocity. Average amplitude of the beats of OKN (Fig. 4 closed circles, and Fig. 5) also increased with increases in drum velocity. Curves for quick phase velocity (Fig. 4, open triangles, and Fig. 5) roughly paralleled those for average amplitude at drum velocities up to 90 /sec. This might be expected since there tends to be a linear relationship between the two at lower amplitudes in monkeys as in humans (Dodge & Cline, 1901; Goto *et al.*, 1965; Cohen *et al.*, 1969).

The frequency of OKN (Fig. 4 open circles, and Fig. 5) also rose with increases in drum velocity. It varied between 1.5 and 4 beats/second. The average frequency of horizontal OKN or caloric nystagmus generally did not exceed 3 beats/sec in the normal monkey whatever the stimulus. When testing with 8 stripes for each 360° of rotation, the frequency of OKN (open circles, Fig. 4 *A*, *B*) was higher at lower drum velocities than the actual number of stripes which passed the eyes (dotted line, "Frequency"). This suggests that the animal was responding more to the velocity of the drum than to the number of stripes which were passing the eyes. This is

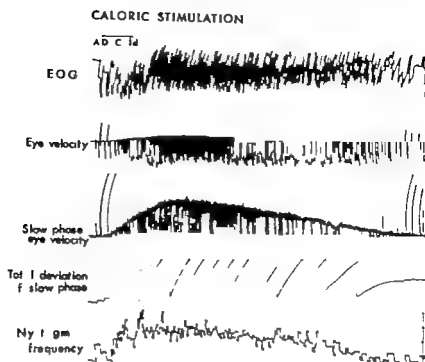


FIG. 6 Caloric nystagmus of a normal monkey induced by 10 seconds of irrigation of the right ear with cold water (27.5°C). The period of stimulation is shown by the horizontal bar between the first and second tracings. The lights were extinguished after the end of caloric irrigation and the rest of the record was taken in darkness. Calibrations as in Fig. 1 except that the vertical bar for the slow phase eye velocity trace represents 60/sec.

in agreement with findings in other studies in animals and man (Gruttner 1939; Honrubia *et al.* 1967).

When the number of stripes in the drum was doubled, maximum slow phase eye velocity did not substantially change (Fig. 4C,D). Frequency of nystagmus increased slightly at higher speeds of drum rotation but not in direct proportion to the increase in the number of stripes. The amplitude of beats tended to be slightly lower when the number of stripes was doubled. There was no consistent change in total deviation.

### Caloric Nystagmus

As in humans (Jongkees & Philipzoon, 1964), 10–15 seconds of stimulation are sufficient to induce 2 to 3 minutes of brisk caloric nystagmus in the monkey. In the example shown in Fig. 6 the eyes initially beat to the left of the midline, i.e. on the quick phase side. 15 seconds later the beating field was across the midline. The tendency of the eyes to beat on the quick phase side of the midline during OKN has been described in man by Gruttner (1930) and Mackensen & Uber (1960). Recently Hood (1967) noted that the eyes also beat primarily on the quick phase side during caloric nystagmus in man. The significance of this phenomenon is not known. This tendency was marked during the nystagmus of Fig. 3 and was present briefly in the initial portions of the nystagmus of Figs. 1 and 5. For the

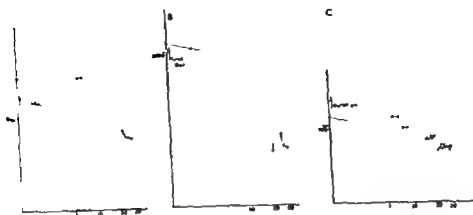


FIG. 7. Graphs showing the decline in various parameters of the oculometer response of normal monkey to repeated caloric testing. The stimulus was 18 cc of water at 27.5°C delivered at the same rate over 18 seconds. Fifteen minutes were allowed to elapse between trials. Trials related sequentially on the abscissa on logarithmic scale. The scale of the ordinate is linear. The ordinate in A is maximum slow phase velocity of the peak of the caloric response. In B the ordinate is total deviation of the eyes in response to the stimulus, and in C duration of the caloric response.

most part, however during both the OKN and caloric nystagmus shown in Fig. 1 and 3 the eyes beat across the midposition.

At the peak of the caloric response (Fig. 6) the beats of nystagmus were about 30 in amplitude, the maximum slow phase velocity was about 105 / sec, and the average frequency was about 3 beats per sec. The amplitude and maximum quick phase velocity of this nystagmus were relatively constant for almost 60 sec during the middle of the response. Frequency had some tendency toward culmination (Torok, 1957) but was relatively constant at between 2 and 3 beats/sec for about 30 seconds during the middle of the response. Maximum slow phase velocity continuously declined after reaching a peak about 30 seconds after the onset of stimulation. The eyes deviated about 6300 in response to the stimulus. Secondary phases of caloric after-nystagmus were commonly present.

In monkeys as in humans, cats and dogs (Crampton & Schwam, 1961; Collins & Updegraff 1966) there was a striking response decline to caloric stimulation. This is shown graphically in Fig. 7. Response decline usually began after several trials for each of the parameters of the nystagmus which were measured and appeared to fall exponentially. Brown & Marshall (1967) have noted the exponential nature of response decline curves in cats. The slope was greatest for maximum slow phase velocity (Fig. 6A) and total deviation of the eyes in response to the caloric stimulus (Fig. 6B). If these curves were extrapolated, they would cross zero at about 110 trials. There was also a steady decline in the duration of the induced nystagmus (Fig. 6C) but the slope of the response decline curve was shallower cross-

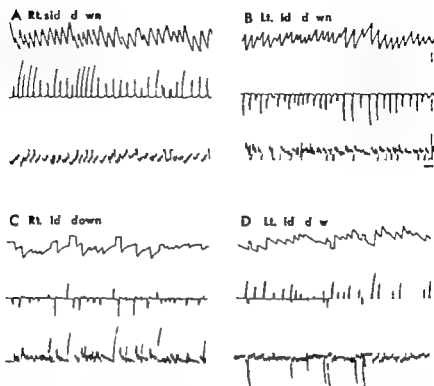


FIG. 8 Positional alcohol nystagmus (PAN) of a normal monkey. The top trace in A-D is the EOG recorded with RC-coupling (3-second time constant); the second trace is the differentiated EOG and the third trace shows slow phase velocity. Rectification was artificial in this case. A and B were recorded 30 minutes after ingestion of alcohol and C and D were recorded 5 hours later. The time base shown by the horizontal bar under B represents 2 seconds. Calibration is in Fig. 1.

ing zero at about 700 trials. Differences in slopes of response decline curves for total deviation and duration of caloric nystagmus also appear to be present in data of Capps & Collins (1965) and Collins & Updegraff (1966) on cats and dogs.

### *Positional Alcohol Nystagmus (PAN)*

Both primary and secondary positional alcohol nystagmus (PAN I and PAN II) were induced in the monkey. PAN I was present in every animal tested, but PAN II was not constantly found. As in humans (Aschan *et al.*, 1958; Bergstedt, 1961) the quick phases of PAN I were geotropic (Fig. 8A, B) and of PAN II were apogeotropic (Fig. 8C, D). In most animals PAN I developed about 20 minutes after ingestion of alcohol and persisted for about one and one half hours. When PAN II occurred, it followed PAN I after a nystagmus free interval of from two to four hours. The nystagmus of PAN II reached a peak about 6 hours after ingestion and lasted for more than 4 hours. PAN II has not been found in cats (Bergstedt, 1961; Jongkees & Philippszoon, 1964) but has been reported in some white rabbits (Nohara, 1966).

## DISCUSSION

Anatomically the structure of the oculomotor apparatus of monkey is close to that of man. Data in this report show that nystagmus of the rhesus monkey is similar in many respects to that of humans. In both monkey and man the best pursuit velocities are about 45 /sec (Robinson, 1965; Fuchs, 1967) while maximum following during slow phases of OKN is about 90 /sec (Gruttner 1939 Ueda & Suzuki, 1965) Maximum quick phase velocities during caloric and optokinetic nystagmus are also comparable in monkey and man (Holke, 1959 Mackensen & Schumacher 1960) as are positional alcohol nystagmus and response decline on repeated caloric stimulation. It is likely that results of oculomotor testing in monkeys can be correlated rather closely with test findings in humans. Changes in eye movements after lesions of the oculomotor system also appear to be similar in monkey and man (Cohen *et al.*, 1968)

In both monkey and man the eyes are able to follow moving targets faster during OKN than during pursuit movements. Theoretically both pursuit movements and slow phases are induced by the same neural organizations (Rashbass, 1961) It is possible that a warm up period is necessary before the slow phase or pursuit system can move the eyes faster than 45 /sec It is of interest that the eyes often move faster than the stimulus at some time during the slow phases and that the velocity of the slow phases is generally not constant. Perhaps the high initial velocity of slow phases is used to overtake the stimulus so that the eyes can approximate the velocity of the target more easily

The major difference in the oculomotor system of monkey and man appears to be in OhAN. OhAN of long duration is sometimes found in humans with labyrinthine or oculomotor system lesions (Hirakae *et al.*, 1963) However in the normal subject OhAN is brief (Gruttner 1939) or cannot be elicited at all. In the monkey OhAN was found in every animal which had OKN and persisted for many seconds. There was no sensory input during OKAN and the response must have been entirely due to activity present within the central nervous system at the end of OhN. That is, neural organizations responsible for OhAN must have stored activity during OKN which was discharged in a regular fashion over the next minute or minute and a half. The neural substrate of OKAN is not known but probably in olives the reticular formation. If the pons and mesencephalon. Long-lasting defects in OhAN occur after lesions in these regions (Shanzer *et al.* 1968 Cohen *et al.* 1968)

A variety of parameters of OhN have been measured. Since slow phase velocity bears the closest functional relationship to the visual input, it should be the parameter of most value in input-output studies (Henriksson, 1966) Indeed maximum slow phase velocity showed the smallest variation of any of the parameters of OhN and was directly related to the stimulus over wider ranges than any other parameter. Only if maximum slow phase

velocity of individual beats of nystagmus is measured, can it be determined whether the eyes are able to approximate the velocity of a moving target.

Frequently however the maximum slow phase velocity of any beat was reached only momentarily. Consequently a more general measure of slow velocity over a longer period of time would appear to be helpful in determining the quality of the response. This is particularly true when the stimulus and/or the oculomotor response vary as during pendular rotation (Setoguchi & Suzuki 1965) the OKP test (Suzuki & Komatsuzaki, 1962) or caloric stimulation, or during spontaneous nystagmus. Because the velocity of individual slow phases varies a great deal, mean slow phase velocity of individual beats is not easy to determine. Total deviation on the other hand gives some measure of mean slow phase velocity over time (Jung & Toennies, 1948) and can easily be determined electronically. Total deviation also tended to be linearly related to drum velocity of up 90 /sec.

In contrast to maximum slow phase velocity and total deviation which appeared to be linearly related to the velocity of the optokinetic stimulus within limits other parameters such as amplitude, maximum quick phase velocity and frequency had a more complex relationship to the OKN stimulus. There was considerable variability in amplitude of beats of nystagmus and in its derivative, quick phase velocity. Nevertheless these parameters are probably the most sensitive measure of the integrity of the rapid eye movement mechanism. Limits of frequency are probably set by the characteristics of the nystagmus mechanism (Cohen *et al.*, 1965) and are related both to the number of stripes passing the eyes in the monkey and to background illumination (Valciukas & Pasik, 1968). Eye position probably also affects frequency and amplitude of OKN, frequency being higher and amplitude lower when the eyes are beating on the quick phase side of the midline (Mackensen & Uber 1960). Changes in frequency amplitude and quick phase velocity of caloric nystagmus and OKN are characteristic after lesions of the pontine reticular formation (Cohen *et al.*, 1968).

Of the other parameters of nystagmus, duration of caloric nystagmus has probably been studied most extensively. It is not much affected by central nervous system lesions (Henriksson, 1956; Jung & Kornhuber 1964; Cohen *et al.* 1968) but has proven to be of value in diagnosis of peripheral labyrinthine lesions (Cawthorne *et al.* 1956). Duration of OKN is arbitrarily controlled but duration of OKAN may be prolonged after peripheral labyrinthine lesions (Kirikae *et al.*, 1963). Since each parameter of nystagmus tends to increase as the stimulus becomes more intense any or all can give some measure of the intensity of the response (Holke, 1959; Torok & Derbyshire, 1968). However it is likely that each parameter has a slightly different contribution to make in diagnosis. When the oculomotor changes produced by discrete lesions of the central and peripheral nervous system are quantitated and known, the diagnostic value of electrooculography should be considerably enhanced.



## ACKNOWLEDGMENT

We thank Mr Edward Murray and David Borras for assistance and the Officer Dixon of Beckman Instruments for supplying couplers used in recording, differentiating, and integrating nystagmus.

## ZUSAMMENFASSUNG

Horizontaler optokinetischer Nystagmus (OKN) kalorischer Nystagmus und alkoholischer Stellungsnystagmus (PAN) des Reassaffen (*Macaca mulatta*) wurden mit einem Elektrookulographen untersucht. Der OKN des Affen erscheint im wesentlichen ähnlich dem des Menschen, obgleich in der optokinetische Nach-Nystagmus mehr ausgeprägt beim Affen ist. Von den gemessenen Werten des OKN stand die Geschwindigkeit der langsamen Phase in engerer Beziehung zur optokinetischen Reizung. Der kalorische Nystagmus ist beim Affen leicht induzierbar und bei dieser Gruppe war eine Abnahme des Nystagmus bei wiederholten Versuchen zu erkennen. Die erste Phase des alkoholischen Stellungsnystagmus war in allen untersuchten Tieren vorhanden und die sekundäre Phase in einigen der Versuchstiere. Die Angaben weisen darauf hin, dass die okulomotorischen Ergebnisse der Versuche beim Affen wahrscheinlich mit einem gewissen Vergleich beim Menschen angewandt werden können.

## REFERENCES

- ANDERSSON, C. I., HALL, G. C., and CROOK, E. C., 1960 *The comparative anatomy of the cranes system of vertebrae including man*. Hafner Publishing Co. New York.
- ANDERSSON, G., BEHREND, M., and SÄLLER, J. 1958 Nystagmography *Acta Otolaryng.* (Stockh.) Suppl. 129.
- BEHREND, M., 1961 Studies of positional nystagmus in the human centrifuge *Acta Otolaryng.* (Stockh.) Suppl. 185.
- BROWN, J. H., and MAREMALL, J. E. 1967 Drug control of arousal and nystagmic habituation in the cat *Acta Otolaryng.* (Stockh.) 81-135.
- CARR, M. J., and COLLINS, W. E., 1963 Effects of bilateral caloric habituation on vestibular magnification in the cat *Acta Otolaryng.* (Stockh.) 59-811.
- CHANDLER, T. D., M. R., HALL, C. B., and HODG, J. D., 1956 The investigation of vestibular function *Br J Med Biol* 12-131.
- COHEN, R., ALBERT, J., KOMATSU, K., and HALL, L., 1969 Effects of light and labyrinthine on horizontal saccadic eye movements of the rhesus monkey *Neurology* (in press).
- COHEN, R., KOMATSU, K., and HALL, M. B., 1968 Electrooculographic syndromes in monkey after posterior colliculus transection lesions. *Arch Neurol* 18-78.
- COHEN, R., STALL, J., and HALL, M. B., 1963 Nystagmus induced by electric stimulation of ampullary nerves. *Acta Otolaryng.* (Stockh.) 60-122.
- COLLINS, W. E., and HODG, R. H. 1962 Amphetamine, arousal, and human vestibular nystagmus *J Pharmacol Exp. Ther.* 132, 129.
- COLLINS, W. E., and LORAN, B. P. 1966 A comparison of nystagmus habituation in the rat and dog. *Acta Otolaryng.* (Stockh.) 63-19.
- CROOK, G. H., and SCHWAB, W. J. 1961 Effects of arousal reaction on nystagmus habituation in the cat *Am J Physiol* 200-25.

- DODGE, R., and CLINE, T., 1901 The angular velocity of eye movements. *Psychol Rev* 8, 145
- FUCHS, A F 1967 Saccadic and smooth pursuit eye movements in the monkey. *J Physiol* 191 809
- GOTO, K TOKUMASU K., and COHEN, B 1968: Return eye movements, saccadic movements, and the quick phases of nystagmus. *Acta Otolaryng* (Stockh.) 65, 426.
- GRUTTMAN, R., 1939: Experimentelle Untersuchungen über den optokinetischen Nystagmus. *Z Sinn sphysiol* 65 1
- HENNINGSON N G 1935: An electrical method for registration and analysis of the movements of the eyes in nystagmus. *Acta Otolaryng* (Stockh.) 45 25.
- 1936: Speed of slow component and duration in caloric nystagmus. *Acta Otolaryng* (Stockh.) Suppl 125
- HONNIGER, V SCOTT B J and WARD, P H 1967 Experimental studies on optokinetic nystagmus. *Acta Otolaryng* (Stockh.) 64 385
- HOOD, J D 1967 Discussion on optokinetic nystagmus in normal subjects. In *Neurotic kinesthetic and vestibular mechanisms* (ed. A. V S de Reuck and J Knight) p. 213. Little, Brown & Co Boston
- JONGHAAS, L B W and PHILIPPOUX, A J 1964 Electronystagmography *Acta Otolaryng* (Stockh.) Suppl 189
- JUNG, R and KORNHUBER, H H., 1964: In *The oculomotor system* (ed. M B. Bender) p 423. Harper (Hoeber) New York.
- JUNG, R., and TOMMERS, J F 1948 Die Registrierung und Auswertung des Drehnystagmus beim Menschen *Klin. Wschr* 26 513.
- KIKUKAWA, I SUZUKI, J and TOKUMASU K., 1963: Spontaneous nystagmus as a sign of clinical significance. *Acta Otolaryng* (Stockh.) Suppl. 179 86
- KOMATSUZAKI, Y 1959 An observation on the eye speed of nystagmus. *Acta Otolaryng* (Stockh.) 59 377
- KRUGER, H P and BENDER, M B 1958 Optokinetic after nystagmus in the monkey *Electroen. ph. Clin. Neurophysiol* 3 97
- LANGER, G 1967 Die normale vestibuläre Erregbarkeit von Affen (*Macaca rhesus*) im Drehversuch der elektronystagmographischen Abteilung *Acta Otolaryng* (Stockh.) 61 119
- MACLEWSEN, G and SCHUMACHER, J 1960 Die Geschwindigkeit der raschen Phase des optokinetischen Nystagmus v Graef *Archiv für Ophthalmologie* 162 400
- MACLEWSEN, G and UHKE, J 1960: Untersuchungen zur Physiologie des optokinetischen Nystagmus. v Graef's *Archiv für Ophthalmologie* 161 590
- MORIMOTO, M., MIZUKOSHI, K., OTSUKI, T IKEDA, S KATSUMI, Y 3 SAKI, T and HONDA, Y 1963 On the secondary phase of nystagmus. *Acta Otolaryng* (Stockh.) Suppl 19 32.
- NOHRA, K., 1966 Influence of alcohol administration upon labyrinthine function in rabbits *J Tokyo Woman Med College* 36 240
- RASHBASS, C., 1961 The relationship between saccadic and smooth tracking eye movements. *J Physiol* 139 326
- ROBINSON, D A 1965 The mechanics of human smooth pursuit eye movement *J Physiol* 180 569
- SETOGUCHI, J and SUZUKI, J 1963: Pendular rotation test. Its clinical significance. In *Proc Int symposium on vestibular and oculomotor problems* p 189 Nippon Hoechst Ltd, Tokyo
- SHANZER, S and BENDER, M B., 1959 Oculomotor response on vestibular stimulation in monkeys with lesions of the brainstem. *Brain*, 82 669
- SHANZER, S TAKAGI, P KRUGER, H P and BENDER, M B 1958 Defects in optokinetic after-nystagmus in lesions of the brainstem *Ann J Physiol* 193 419
- SHIBATA, R and LEE, J C., 1961 *A stereotaxic atlas of the monkey brain* University of Chicago Press, Chicago

- SHIMIZU, J. and KOMATSUZAKI, A. 1962 Clinical application of optokinetic nystagmus. *Acta Otolaryng* (Stockh.) 54: 49.
- TOMON, Y. 1967 The culminative phenomenon and frequency pattern of thermic nystagmus. *Acta Otolaryng* (Stockh.) 88: 530.
- TOMON, Y. and DEKRA-SHIMIZU, A. J. 1968 Computation of the nystagmogram. *Acta Otolaryng* (Stockh.) 65: 70.
- YEDA, R. and SHIMIZU, J. 1965 Studies on the yaw speed during voluntary ocular movements and induced nystagmus in normal test subjects. In *Proc. of symposium on vestibular and ocular problems*, p. 89 Nippon Hoechst Ltd., Tokyo.
- VALCHUK, J. A. and PARIKH, P. 1968 Optokinetic nystagmus (OKN) stimulus-response relationships in normal monkeys. *Fed. Proc.*, 27: 638.

B. Cohen, M.D. Neurophysiology Laboratory  
 Atrium 710 The Mount Sinai Hospital, 1 East  
 100th Street New York, N.Y. 10029 U.S.A.

Received July 1 1968

## RENAL ADENOCARCINOMA WITH METASTASES TO THE THYROID GLAND

S FRIBERG Jr and J KINMAN

*From the Department of Radiopathology and the Department of Otolaryngology  
Karolinska Sjukhuset Stockholm Sweden*

Four cases of renal adenocarcinoma with metastases to the thyroid gland are reported. In two cases the metastases developed almost simultaneously with the primary tumor and in the other two cases there was an interval of 12 and 16 years, respectively from nephrectomy to the appearance of metastases. Some of the peculiar features of renal adenocarcinoma are briefly discussed, as well as the incidence of metastases in general to the thyroid gland, their diagnosis and principles of treatment. These four cases bring to 48 the total number of published cases of renal adenocarcinoma with metastases to the thyroid gland that we have found in the literature.

Renal adenocarcinoma<sup>1</sup> in humans is a neoplasia with some unusual features. This tumor has lately been called "a clinical challenge" (Hewitt, 1967) and it has been stated that "malignant renal tumors should be classed with syphilis and tuberculosis as among the great mimics encountered in clinical medicine" (Creedy, 1935). Some of the main reasons for the uniqueness of renal adenocarcinoma can be summarized in four groups:

1. The symptoms of the primary tumor are various, often late and sometimes completely absent (Arner & von Schreeb, 1966; Böttiger *et al.*, 1966).

2. Any organ can be the site of secondary deposits. This may be explained in part by the rich vascular supply to the kidney and the tumor (Mostofi, 1967).

3. The time of development of metastases is even more unpredictable than in other malignancies, although there exists a fair correlation between the clinical signs, morphology of the tumor and staging of the disease on one hand and prognosis on the other (von Schreeb, 1967).

4. Both the primary tumor and the metastases have been claimed to show spontaneous regression (for discussion and reviews of the literature see Goodwin *et al.* 1967 and Markewitz *et al.* 1967).

The most common sites for secondary deposits from renal adenocarcinoma are Lungs, lymph nodes, skeleton and adrenals (Riches *et al.*,

Synonyms: Grawitz tumor, hypernephroma, clear cell adenocarcinoma, granular cell carcinoma, transitional but a few

1951 Lucké & Schlumberger 1957 Mellicow & Uson, 1960) but as men know any organ can be involved and any clinical specialist may encounter a metastasis in "his" field.

Thus, the otolaryngologist may see secondaries from a renal adenocarcinoma in the paranasal sinuses (Hamberger 1943 Eneroth *et al* 1961) in the thyroid gland, or rarely in the mandible (Leahien, 1966) tongue, tonsils, or larynx.

In the thyroid gland, reported metastatic lesions from renal adenocarcinoma are rare. The first published case seems to be the one discovered at autopsy by Lubarsch in 1894. In 1954 Bruce & Michie published a summary of 22 previously reported cases and added two of their own. To our knowledge, 20 further cases have since been reported (Illyés & Gerlei, 1954 (1 case) Hartmann, 1958 (1) Lange, 1960 (1) Mellicow & Uson, 1960 (2) Helmann, 1961 (2) Shimaoka *et al* 1962 (6) Wychulis *et al* 1964 (4) Freund, 1965 (1) Burge & Blalock, 1967 (1) Crocker 1967 (1)). Of these 44 cases, 16 have been detected at autopsy and 28 during the life-time of the patients.

In the Department of Otolaryngology and from the files of Radiumhemmet, Karolinska Sjukhuset, we have collected four cases of renal adenocarcinoma with metastases to the thyroid gland.

### Case Reports

#### Case 1

The patient was an 88-year-old man who presented in January 1951 an acute onset of severe back pain on the right side in connection with vomiting and dark coloured urine. Renal angiography demonstrated an orange-sized tumor in the lower part of the right kidney containing several pathological vessels. The sedimentation rate (SR) was 25 mm per hour.

During this admission nephrectomy on the right side was performed. The lower part of the right kidney was occupied by an orange-sized tumor. It was a circumscribed renal adenocarcinoma without any apparent infiltration of the renal pelvis or vessels. The carcinoma was moderately well differentiated and classified as malignancy grade II A according to Arner (Arner *et al* 1965). The tumor was radically extirpated.

The patient remained asymptomatic for 16 years, but in the course of a check up at an eye clinic in February 1967 a tumor unnoticed by the patient, was detected in the lower right part of the neck. A needle biopsy from the tumor contained tumor cells with abnormal nuclei most compatible with a metastasis from a renal carcinoma. Its tracing showed normal values, but the scintigram demonstrated a defect in the tumor area (Fig. 1). Radiographs of the chest and vertebral column were normal. The patient was admitted to the Department of Otolaryngology in May 1967 in good general condition. He had a rather soft, non-tender mass, the size of a man's fist, in the lower right part of the neck (Fig. 2). Laboratory examinations



FIG. 1



FIG. 2

FIG. 1 Scintigram in case 1, an 88-year-old male operated 16 years previously for a renal carcinoma on the right side. A large defect is demonstrated in the lower part of the right thyroid lobe.

FIG. 2 The clinical finding in case 1 was a fist-sized, firm, indurated tumor corresponding to the lower part of the right thyroid lobe.

showed hemoglobin (Hgb) 13.4 g/100 ml, SR 22 mm per hour, white blood cell count 3400, serum creatinin 1.0 mg/100 ml. Three days after admission the thyroid gland was exposed via a collar incision, the right recurrent nerve was isolated and a hemithyroidectomy including the tumor was performed on the right side.

The histopathological finding was a radically extirpated metastasis from a renal carcinoma to the thyroid. It was classed as malignancy grade II A, same as the primary tumor.

At the last physical examination in August 1967 there were no signs of recurrence of the tumor or further metastases.

### Case 2

The patient was a 46-year-old man. In December 1966 he noted pain in the groin. Urography in January 1967 showed an expansive process in the right kidney, and renal angiography demonstrated a densely vascularized tumor (Fig. 3). No metastases could be detected, and the left kidney was roentgenographically normal. The SR was 66 mm/hour and Hgb 10.3 g/100 ml. A needle biopsy from the tumor was attempted, but no tumor cells were obtained.

Nephrectomy on the right side was performed in March 1967. The upper pole of the kidney was occupied by a grapefruit-sized tumor which was



Fig 2. Renal angiography (case 2). During the vascular phase several tortuous, pathological vessels were demonstrated (arterial glomeruli with areas of very poor arterial supply).

ally removed. On the abdominal surface of the diaphragm a hazelnut-sized tumor was detected and removed.

The tumor was a renal adenocarcinoma with the maximum diameter of 11 cm. The tumor removed from the diaphragm was a metastatically involved lymph node. Both were classified as malignancy-grade II B (Fig 4). No invasion of the vessels of the kidney could be detected and the carcinoma as well as the metastasis had apparently been removed in toto.

In April 1967 the patient was admitted to Radiumhemmet for postoperative irradiation. On this occasion a plum-sized tumor was observed in the lower part of the right thyroid lobe. The patient stated that he had noticed the swelling right after the renal angiography 3 months previously. A needle biopsy from the tumor contained dense cell-complexes of rather monomorphic cells with pronounced vacuolization in the cytoplasm. The cytological interpretation was that the tumor probably was a thyroid adenoma with regressive changes, but a metastasis from a renal adenocarcinoma could not be excluded.

<sup>131</sup>I tracing showed normal values, but on the scintigram the lower part of the right lobe had a decreased uptake.

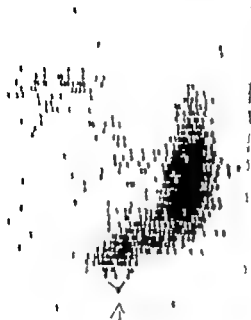


FIG 1



FIG. 2

FIG. 1 Scintigram in case 1 an 58-year old male operated 16 years previously for a renal carcinoma on the right side. A large defect is demonstrated in the lower part of the right thyroid lobe.

FIG. 2 The clinical finding in case 1 was a fist-sized, rather soft, indolent tumor corresponding to the lower part of the right thyroid lobe.

showed hemoglobin (Hgb) 13.4 g/100 ml, SR 22 mm per hour, white blood cell count 3400, serum creatinin 1.0 mg/100 ml. Three days after admission the thyroid gland was exposed via a collar incision, the right recurrent nerve was isolated and a hemithyroidectomy including the tumor was performed on the right side.

The histopathological finding was a radically extirpated metastasis from a renal carcinoma to the thyroid. It was classed as malignancy grade II A, same as the primary tumor.

At the last physical examination in August 1967 there were no signs of recurrence of the tumor or further metastases.

### Case 2

The patient was a 46-year-old man. In December 1960 he noted pain in the groin. Urography in January 1967 showed an expansive process in the right kidney and renal angiography demonstrated a densely vascularized tumor (Fig 3). No metastases could be detected, and the left kidney was roentgenographically normal. The SR was 66 mm/hour and Hgb 10.3 g/100 ml. A needle-biopsy from the tumor was attempted but no tumor cells were obtained.

Nephrectomy on the right side was performed in March 1967. The upper pole of the kidney was occupied by a grapefruit sized tumor which was



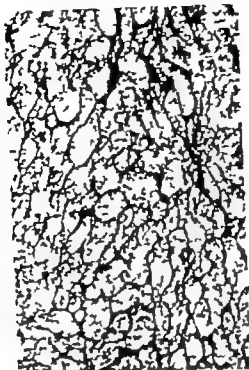


FIG. 6. Larger magnification of the metastasis shown in Fig. 7. The cellular pattern is less polymorphous than that of the primary tumor in Fig. 5, to which it should be compared. H. E. 140.

excised. The pathological examination showed a totally extirpated metastasis from a renal adenocarcinoma. It was more highly differentiated than the primary tumor which explains the discrepancy between the cytological picture (high differentiation) and the histopathological one ("medium high differentiation"). The thyroid parenchyma was otherwise normal (Figs. 4 and 5).

Three months after the operation on the thyroid (8 months after the nephrectomy) there were no signs of recurrence or further metastasis.

### Case 3

This male patient was 38 years old, when, in 1938, he had an acute episode of abdominal pain and blood in the urine. A fist sized mass could be palpated in the region of the left kidney. No other examinations are recorded. The patient was operated on in October 1938 with a nephrectomy on the right side. The kidney was partially occupied by an orange-sized tumor which was totally removed. No metastases could be detected.

The microscopic slides showed a renal adenocarcinoma of malignancy grade II B, without signs of penetration of the renal pelvis or vessels.

The patient remained well and without symptoms until 1950, when he



Fig. 4

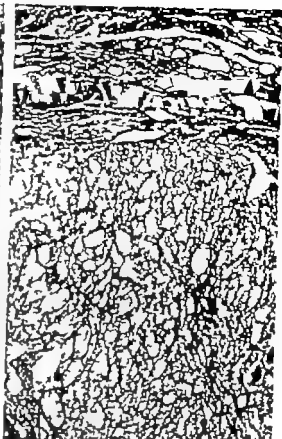


Fig. 5.

FIG. 4 Microphotogram of the primary tumor of case 2, showing the structure and cellular picture of a renal adenocarcinoma, malignant grade II B. Haematoxylin Eosin  $\times 160$

FIG. 5 Microphotogram of the metastasis to the thyroid gland in case 2. In the upper part of the picture is seen thyroid parenchyma in the lower part neoplastic tissue H.E.  $\times 64$

In May 1967 irradiation to the previously operated area was started, but when 4500 r had been given the platelets had decreased to 80 000/cc and irradiation was temporarily discontinued. During the period of irradiation repeated urographies of the left kidney and X rays of the lungs had been normal.

The patient was admitted to the Department of Otolaryngology in August 1967 for surgical exploration of the thyroid region. Physical examination revealed a walnut sized, hard tumor in the right thyroid lobe and laboratory examination showed Hgb 11.2 g/100 ml, SR 58 mm/hour, white blood count 2700/cc with 42% lymphocytes, platelet count 85 000/cc. As the cytologic diagnosis had not been clear-cut, the surgical intervention on the thyroid gland was as much a diagnostic as a therapeutic one.

At operation the thyroid gland was exposed via a collar incision and as the tumor was well circumscribed from the thyroid tissue, it was easily

## DISCUSSION

*Incidence of Metastases to the Thyroid Gland*

The incidence of clinically manifest metastasis to the thyroid gland is much lower than that found in series based on autopsy material.<sup>1</sup> This is to be expected, as the autopsy cases usually present the neoplastic disease in a more advanced stage. Thus, in a series of 30 000 thyroidectomies performed in the Mayo clinic from 1892 to 1943 Long & Black (1943) reported 22 instances of carcinomatous metastasis to the thyroid gland (three of these were from renal adenocarcinomas) and Bruce & Michle (1954) in a series of 1600 thyroidectomies, reported four instances of secondaries (two of these were from renal adenocarcinomas). Series based on autopsies, however, show that the thyroid gland is more frequently the site of metastasis than is usually recognized. In consecutive autopsies on patients with unselected primary cancers, Willis (1931) reported nine instances of metastasis to the thyroid gland out of 170 cases (5.3%). Mortensen *et al* (1956) 18 out of 467 cases (3.9%) and Shimaoka *et al* (1962) 188 in 1909 cases (9.4%). In this last series only nine cases of primary thyroid carcinomas were found, which seems to indicate that in patients with cancer of another organ, metastasis to the thyroid gland occurs about 20 times more frequently than primary thyroid carcinoma.

Most of the reported metastases to the thyroid have occurred in abnormal glands, usually a colloid goiter. This experience is supported by experimental results, because an abnormality which decreases the blood-flow through an organ can enhance the formation of metastasis in two ways: (1) The slow blood-flow facilitates the deposition of tumor cells according to Coogan *et al* (1951) and Fischer *et al* (1967) and (2) a lowered oxygen tension favours the growth of anaerobic i.e. tumor cells. (Warburg, 1926; Bruce & Michle 1954; Lange 1960). In two of our cases the metastasis occurred in abnormal thyroid tissue (cases 3 and 4).

*Diagnosis*

Even if as pointed out by Shimaoka (Shimaoka *et al* 1962) a malignant tumor in the thyroid gland in a patient known to have another cancer is more likely to be metastatic than primary, the possibility that it is a second primary neoplasm should always be considered. Also, in the case where no primary tumor is known, a thyroid nodule could represent metastasis from an undetected cancer. The diagnosis of thyroid tumors is therefore very important and as much information as possible should be gathered preoperatively, for example by I<sup>131</sup>-uptake scintigram and needle biopsy (Einhorn & Franzen, 1962). The cytological differential diagnosis, however, be

<sup>1</sup> Apparently this is not valid for renal adenocarcinoma as stated in the introduction of the paper.

noticed a swelling of the thyroid. He had no signs of toxicity and a consulted doctor diagnosed goiter. As the swelling did not bother the patient, it was decided to leave the thyroid untreated. But the gland continued to enlarge, and in April 1953 a partial strumectomy was attempted. The thyroid and its surroundings turned out to be the seat of tumor growth, however, and it was not technically possible to remove the neoplasia completely. Microscopy revealed metastatic renal adenocarcinoma of malignancy grade II A to a colloid goiter.

The patient was referred to Radiumhemmet for post-operative irradiation. After this treatment the patient remained well and without signs of further metastases until November 1953 when a secondary in the right tibia was discovered. This regressed after radiation and no progression of the disease was noticed until February 1956 when cranial nerve signs developed. The patient died in December the same year with widespread cerebral metastases. No autopsy was performed.

#### Case 4

The patient was a male, who at the time of diagnosis was 68 years old. Since "many years" he had a slowly increasing enlargement of the thyroid gland without signs of toxicity. The lesion had been diagnosed as a non-toxic goiter and no treatment had been given.

In February 1962 the patient noted dark-coloured urine. Laboratory examinations showed Hgb 14 mg/100 ml, SR 15 mm/hour. Renal angiography demonstrated a tumor in the left kidney and also a probable metastasis in the left adrenal. Nephrectomy was performed in February 1962, but the adrenal was not extirpated. The tumor was a renal adenocarcinoma of malignancy grade II A and had been radically excised.

Nine months later the patient was again admitted to the hospital because of compression symptoms from the enlarged thyroid gland. The gland had just before the admission started to grow rapidly and was now interfering with the patient's breathing. X-rays indicated a grossly enlarged and partially intra-thoracic thyroid.  $I^{131}$  tracing gave normal values, but the scintigram did not show any uptake in the intra-thoracic part. During the laboratory investigations the patient developed acute dyspnea necessitating surgical intervention. At operation several tumors were found in the enormously enlarged thyroid, and numerous large, hard lymph nodes were also discovered. As some of the tumor tissue infiltrated in the surroundings, radical removal was not possible.

Microscopy showed metastases to a colloid goiter and lymph nodes from an adenocarcinoma with similar structures and malignancy grade as the patient's renal tumor.

Post-operatively the patient was referred to Radiumhemmet for irradiation therapy. The disease progressed, however, and the patient died in October 1964 with generalized metastases. He was not autopsied.

## REFERENCES

- ÅBERG, O., BILSTEN, C., and von SCHEER, T. 1945 Renal adenocarcinoma. Morphology—grading of malignancy—prognosis. *Acta Chir. Scand.*, 5 ppl. 346.
- ÅBERG, O., and von SCHEER, T. 1946 Renal adenocarcinoma. Symptoms and signs. *Acta Chir. Scand.* 132, 348.
- BACE, C. G., and MICHAEL, W. 1934 Metastatic hypernephroma of the thyroid gland. *Brit. J. Surg.* 42, 237.
- BACE, J. P., and BALOGH, J. B. 1947 Metastatic hypernephroma of the thyroid gland. *Am. J. Surg.* 113, 387.
- BÖTTCHER, L. E., HALLANDER, D., and SCHEER, T. 1946 Renal adenocarcinomas as an accidental finding. *Acta Chir. Scand.* 132, 348.
- COMAY, D. R., DE LOVA, R. P., and MCCARTNEY, M. 1951 Studies of the mechanisms of metastases. The distribution of tumors in various organs in relation to distribution of arterial emboli. *Cancer Res.* 11, 648.
- CHERRY, G. D. 1933 Coexisting clinical manifestations of malignant renal neoplasms. *Arch. Int. Med.* 53, 835.
- COCHRAN, D. W. 1947 *J. Renal Tumors* (ed. S. C. Sommers) p. 212. W. B. Saunders Company, Philadelphia.
- ERIKSSON, J., and FALCK, S. 1962 Thin-needle biopsy of the thyroid gland. *Acta Radiol.* 53, 331.
- ERIKSSON, C. M., MÄRTENSON, G., and TÄRT, A. 1961 Profuse metastases of hypernephroma metastases. *Acta Otolaryng.* (Stockh.) 54, 548.
- FACHNER, E., and FACHNER, E. R. 1949 Experimental evidence in support of the dormant tumor cell. *Science* 109, 918.
- FACHNER, E., FACHNER, E. R., and FEDERMAN, N. 1947 Trauma and the localization of tumor cells. *Cancer* 20, 22.
- FALCK, H. R. 1945 Surgery of the breast and the thyroid gland from other primary malignancies. *Ann. Surg.* 162, 285.
- GARDNER, W. L. 1945 Some influences of hormones on the growth and persistence of transplanted testicular tumors. *Cancer Res.* 5, 497.
- GOODWIN, W. E., MARR, M. M., MARR, J. J., COCHRAN, A. T. R., and MARTIN, D. C. 1947 Under what circumstances does "regression" of hypernephroma occur? *J. Renal Neoplasms* (ed. J. S. King, J.) p. 41. Little, Brown & Co., Boston.
- HÄRDIN, G. 1934 The dormant cancer cell. *Brit. Med. J.* 2, 667.
- HÄRDIN, G. A. 1945 Hypernephrommetastases of the thyroid gland and pheochromocytoma. *Verh. Med. (Stockh.)* 20, 2229.
- HÄRDIN, G. 1948 Über bösartige schilddrüsen primäre Schilddrüsenmetastasen. *Wien. Klin. Wochenschr.* 60, 785.
- HÄRDIN, G. 1961 Secondary hypernephrommetastases of the thyroid gland after nephrectomy. *Verh. Med. (Stockh.)* 61, 732.
- HERRICK, C. B. 1947 *J. Renal Neoplasms* (ed. J. S. King, Jr.) p. 2. Little, Brown & Co., Boston.
- KILB, A. Z., and GRUBB, F. 1934 Hypernephroma (Granula-Tumor) metastatic in the thyroid gland. *Z. Path. Pathol.* 82, 24.
- LAUR, G. 1940 Hypernephrommetastases of the thyroid gland. *München. Med. Wochenschr.* 192, 2157.
- LAUR, H. 1946 Metastatic renal carcinoma of the mandible. *Swedish Tandläkare-Tidskrift* 53, 639.
- LOVE, C. G., and BLACK, B. M. 1945 Metastatic hypernephroma of the thyroid. *Proc. Mayo Clin.* 40, 43.
- LISSNER, H. 1934 Beiträge zur Histologie der von Nebennierentumoren ausgehenden Metastasen. *Arch. Path. Anat.* 133, 149.

tween a thyroid adenoma, a well differentiated thyroid carcinoma and a metastasis from some other well differentiated adenocarcinoma can be utterly difficult (as was demonstrated in our case no 2) Frozen sections during operation can also in the above-mentioned cases, be extremely hard to evaluate (Burge & Blalock, 1967)

### *Treatment*

The principle of removing an organ because of the presence of a metastasis should be applied also to the thyroid, and especially if the primary carcinoma appears to be controlled and there is no evidence of metastasis elsewhere. In the case of a single metastasis simple extirpation seems justified, whereas in the case of multiple metastases, general principles for surgery in thyroid cancers should be applied the exception being, however that radical neck-dissection seldom is indicated. Post-operative irradiation should be considered in the cases where radical removal of tumorous tissue can not be accomplished

### *Clinical Course*

The clinical course of renal adenocarcinoma is relatively often one of slow progression with late development of metastases. In the cases with secondaries to the thyroid, for example, the interval between the detection of the primary tumor and the development of clinical manifest metastasis averaged  $9\frac{1}{2}$  years in the 18 patients, where it was recorded (range 3-23 years) <sup>1</sup> In two of our cases the metastases developed shortly after nephrectomy but in the other two there was a free interval of 12 and 10 years, respectively. Such long intervals illustrate one of the bizarre characters of renal adenocarcinoma, which it sometimes shares with some other and usually hormone-dependent neoplasias (Morton & Morton, 1953). These free intervals have led to the theory of "dormant cells" (Hadfield, 1954) which have received some experimental support (Gardner 1945 Fischer & Fischer 1950). Our two cases were not autopsied, however and a new renal adenocarcinoma in the remaining kidney can therefore not be excluded.

### ZUSAMMENFASSUNG

Es sind vier Fälle mit Adenokarzinom der Niere mit Metastasen in der Schilddrüse gemeldet. In zwei Fällen entwickelten sich die Metastasen gleichzeitig mit dem Primärtumor und in den anderen zwei Fällen bestand ein Intervall von 12 bzw. 16 Jahren von der Nephrectomie bis zum Auftreten der Metastasen. Einige der bizarren Kennzeichen des renalen Adenokarzinoms sind kurz diskutiert worden ebenso das Vorkommen von Metastasen in der Schilddrüse im Allgemeinen ihre Diagnose und die Prinzipien der Behandlung. Diese vier Fälle bringen die totale Anzahl der veröffentlichten Fälle von Adenokarzinom der Nieren mit Metastasen in der Schilddrüse, die wir in der Literatur gefunden haben, auf insgesamt 48.

Figure calculated by the authors from the published papers.

## ELECTRON MICROSCOPIC STUDIES OF THE OTOSCLEROTIC FOCUS

I. G. CHEVANCE, M. BALSLEY JØRGENSEN, P. BRETLAU and J. CAUSSE

*From the Laboratoire de Cytologie Faculté des Sciences Paris France and the University ENT Clinic Rigshospitalet Copenhagen, Denmark*

From electron microscopic studies of otosclerotic tissue fixed immediately after surgical removal of the stapes, we feel we can deduce the following. First Apart from the borderline between focus and normal tissue as seen in the light microscope, there are "microfoci" demonstrable only in the electron microscope. The focus appears to enlarge by fusion of these microfoci. Second Osteoclasts appear to play a substantial role during the so-called phase of resorption. On the other hand, we demonstrated pronounced osteocytic resorption, particularly lysis of collagen being a invariable finding. In relation to these findings, lysosomes were often observed in the osteocytes. The endothelial cells of the capillary network appeared to be normal.

Otosclerosis has not as yet been subjected to thorough study by electron microscopy only a few investigations by this method being on record (Chevance 1962, Reydon & Smith, 1968, Frank *et al.* 1968). This fact is surprising, considering the gaps in our knowledge of this disease especially its cytological aspects. To-day there is relatively ample material for study since surgical removal of the stapes is now practised in most ENT departments. However the explanation of this lack of investigation refers to certain difficulties concerning the properties of the material.

In the first place, the demand for immediate fixation of the removed bony fragment requires a close collaboration between surgeon and cytologist. The surgeon must also indicate where in the stapes or in the removed bony fragment the focus is situated. Lacking a precise orientation of the material during the embedding process, the focus may easily be lost.

Another and perhaps greater difficulty is that the tissue to be studied is bone and without decalcification it is not possible to obtain sections thin enough for cytological study. Without decalcification the presence of calcium would prevent the electron from penetrating the material.

Chevance (1960) suggested using EDTA (ethylene diamine tetraacetic acid) decalcification medium which was applicable to the bony labyrinth. A neutral pH chelating substance was used in the present study. Acid decalcification media are not applicable in electron microscopic studies. The technique will be briefly described below.

The stapes or fragment of the stapes, and in some cases osteoid lamellae removed at operation for tinnitus in Buzlers and in Copenhagen were

- LUCKÉ, B. and SCHLUMBERGER, H. G. 1957 *Tumors of the kidney, renal pelvis and ureter* fasc. 30 AFIP Washington, D.C.
- MARKEWITZ, M., TAYLOR, H. A. and VERKEMA, H. J. 1967 Spontaneous regression of pulmonary metastases following palliative nephrectomy *Cancer* 20 1147
- MELICOW, M. M. and USOW, A. G., 1960 Nonurologic symptoms in patient with renal cancer *J.A.M.A.* 172 146
- MONTESANO, J. D., WOOLNER, L. B. and BENNETT, W. A. 1956 Secondary malignant tumors of the thyroid gland *Cancer* 9 306
- MORTON, J. J. and MORTON, J. H. 1953 Cancer as a chronic disease. *Ann. Surg.* 137 633.
- MOSTOFI, F. K., 1967 In *Renal neoplasia* (ed. J. S. King, Jr) p. 41 Little Brown & Co., Boston
- RICHES, E. W., GRIFFITHS, I. H. and THATCHER, A. C. 1951 New growths of the kidney and ureter *Brit. J. Urol.* 23 297
- VON SCHNELLEN, T. 1967 Renal adenocarcinoma—clinical problems with special reference to pre-operative evaluation of malignancy *Acta Chir. Scand.* 5 ppl 381
- SHIMAZAKI, K., SOMAI, J. E., and PICKERS, J. W. 1962 Metastatic neoplasms of the thyroid gland. *Cancer* 1 557
- WARBURG, O. 1926 *Über den Stoffwechsel der Tumoren* *Abhandlungen des Kaiser Wilhelm Institut für Biologie* ed. Otto Warburg Berlin.
- WELLS, R. A. 1931 Metastatic tumors in the thyroid gland *Am. J. Path.* 187
- WICHULSKA, A. H., BEARR, O. H., and WOOLNER, L. H. 1964 Metastasis of carcinoma to the thyroid gland. *Ann. Surg.* 160 169

S. Friberg M.D.

Dept. of Radiopathology Karolinska Sjukhuset  
S-10401 Stockholm Sweden

Received June 13 1968





FIG. 1. Light micrograph showing "osteoid lamellae" (OL)—small area of formation of bone lamella surrounding the osteocytes (O). These bony structures are located under the macrophages (F). 800X.

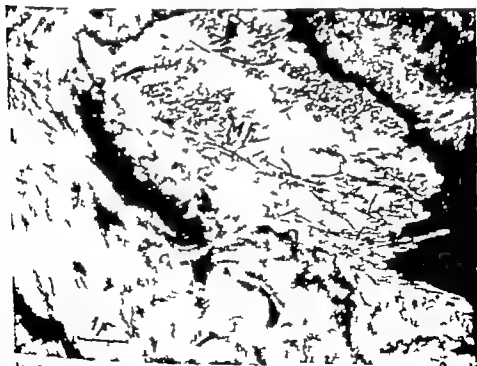


FIG. 2. Electron micrograph showing "macrophage" (MF) with absence of collagen fibers. Newly formed bones. 23,000X.

fixed immediately after removal in a solution of 25% glutaraldehyde mixed with cacodylate buffer pH 7.2

After 24 hours at 4 C in this solution the preparations were decalcified in a 4.5% solution of EDTA 2 Na adjusted by NaOH to pH 7.2. The decalcification in this solution at 4 C takes 2-4 days. The decalcified preparations may be stored in the cacodylate buffer for several weeks.

After rinsing in Tyrode's solution and post fixation in 4% osmic acid,  $\text{OsO}_4$ , the preparations were dehydrated and embedded in Epon as advocated by Luft (1961). Epon solution A and B is mixed at the ratio 7:3.

From the preparation blocks, survey sections  $1/2-1 \mu$  thick were made for light microscopic studies. These sections were stained with 1% toluidine blue in a saturated solution of borax. Thereafter small pyramids were made from the transitional zone between otosclerotic and normal bony tissue. Ultrathin sections of these pyramids were collected on coated copper grids and contrasted with uranyl acetate and basic Pb citrate for 1-5 min at room temperature.

The electron microscopic studies were performed in Paris with a type Hitachi microscope and in Copenhagen with a Philips 200.

## RESULTS

As the pictures obtained by these technical procedures show certain changes, the point is (as in all electron microscopic studies of abnormal tissues) to make sure that such changes are not artefacts due mainly to the process of decalcification but really represent the cytological appearance of the otosclerotic focus.

This very important point was solved by a close collaboration between

The electron microscopic studies were performed in Paris with a type surgeon and cytologist Causse (1960) has described as "osteoid lamellae" small new formations of bony lamellae found in cases of developing otosclerosis in the very limited area between the fissula ante fenestram and the anterior pole of the stapes. These very thin bony structures are arranged like the tiles on a roof beneath the mucosa of the abovementioned zone. They seem to creep from the fissula to the stapes which they eventually surround and block.

We first studied these abnormal structures which are, if not constant then very common phenomena during the development of the disease. They always present themselves as new developing otosclerotic bone so thin and so loosely bound to their organic matrix that they can be cut without decalcification, unlike the hard and brittle bone of the stapes. Accordingly these "osteoid lamellae" may be considered perfect reference material in comparing with the decalcified stapedial bone (Fig. 1).

The electron microscopic studies of the otosclerotic focus may conveniently be divided into two parts: (a) the bony tissue (b) the cells.



Fig. 1. Electron micrograph showing osteoid lamellae (OL)—small new formations of bone lamellae, surrounding the osteocytes (O). These bone structures are located under the surface (fibroblasts, F)  $\times 8000$ .

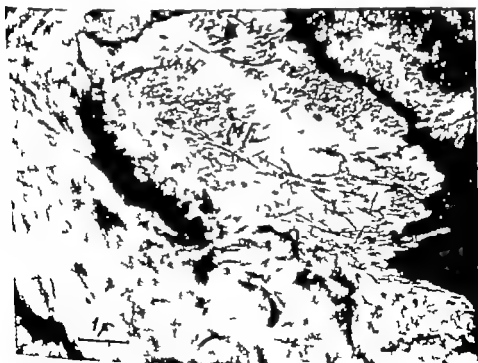
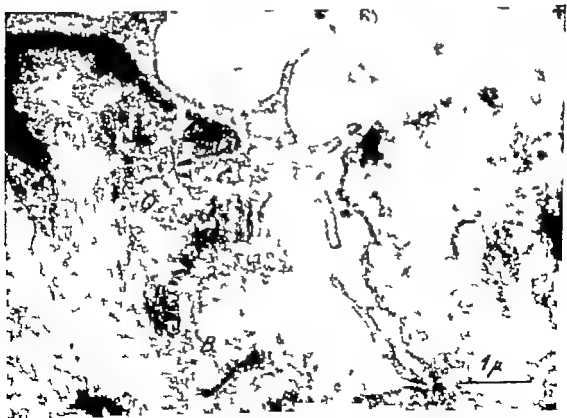


Fig. 2. Electron micrograph showing "microfocus" (MF) with bone of collagen fibers in newly formed bones.  $\times 23,000$ .



a



b



FIG. 2. (a) Electron micrograph showing osteocyte (O) and its lacuna (L) on the way of lysis. Not the rarefaction of the normal cell for organelles, especially the lack of mitochondria. The cell is shrunken. 10,000. (b) Electron micrograph showing more advanced stage in the lysis of the osteocyte (O). The cell is shrunken and its border (B) is in contact with the border of the lacuna. 10,000. (c) Electron micrograph showing an osteocyte undergoing lysis with nuclear fragmentation and cytoplasmic vacuolization. 10,000.

### The bony tissue

After decalcification, this tissue presents itself as being made up mainly of a multitude of interwoven collagenous bundles, so entangled and intricate that a systematic description is impossible. It is in fact what the German histologists called *Faser knochen*. No other bone shows such strong collagenous matrix. It is not possible to distinguish anything in the way of a laminar system. Two facts should be emphasized:

1. Cartilage cells are never found within the limits of an osteosclerotic focus.
2. A striking finding in the collagen tissue, not suspected from the light microscopic studies, is a multitude of "microfoci" in the immediate neighbourhood of the large more evident ones (Fig. 2). The borderline between normal and diseased tissue is always very sharp with no fringe in the periphery of the foci, not even at high magnification.

The entire picture gives the impression that the disease is "splashing" around from active centres.



FIG 4 Electron micrograph showing a normal aspect of a capillary (C) running through an osteocytic focus (OF) 15,000

Regardless of their size, the foci generally present themselves as gaps or empty zones, but sometimes the process of destruction is not complete and the collagen bundles may be observed in the process of lysis. The individual bundle gets thinner losing its normal periodic striation at some distance from the end of the fibre.

### *The cells*

The cellular aspects are not so uniform: lysis predominating. Not uncommonly there are lacunae in the tissue outlining the general shape and size of an osteocyte which has totally disappeared. This finding has to be studied as a function of the surrounding tissue. If it seems normal the finding may be interpreted as the remnant of an osteocyte whose lacuna has filled with a labile calcium deposit which has disappeared in the course of decalcification. Such a finding is not of immediate pathological significance.

On the other hand, if the surrounding tissue shows one or more active osteosclerotic foci in the immediate vicinity the lytic process may be considered an active one. If so however the surrounding collagenous structures display discrete signs of changes, chiefly in the form of localized rarefaction or irregularity of the cell lacuna.

More interest attaches to the finding of cells in the process of lysis (Fig. 3a, b, c). The first stage is loss or rarefaction of the normal cellular

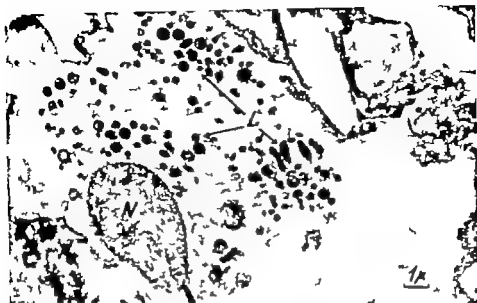


Fig. 3. Electron micrograph showing an osteocyst with its nucleus (N) and the cytoplasmic number of round vacuoles of different sizes containing osmophilic material (lysosomes) are prominent features (L).  $\times 10,000$

organelles. The most striking feature is rarefaction of the mitochondria and shrinkage of the cell, its borders being no longer anywhere in contact with the borders of its lacuna. The second stage is partial lysis, nuclear fragmentation and cytoplasmic vacuolization.

These appearances call to mind first the possibility of artefacts, but for two reasons we believe that they do not represent artefacts.

(a) Many times we have observed, in the same electron microscopic picture a normal cell and nearby a cell in the process of lysis, and an artefact due to fixation cannot affect one cell and not another only about 50 square  $\mu$  apart.

(b) Osteoid lamellae which we have studied without decalcification have shown essentially the same process of lysis.

These findings are highly suggestive of an initial role played by the cells in otosclerosis. Incidentally our findings have not confirmed the theory advanced by some authors that the capillaries are important factors in the growth and spread of the disease. Fig. 4 shows a perfectly normal capillary running through an active otosclerotic focus.

It is striking that we have never made any findings which might suggest an active osteoclastic cell.

Of course, a negative finding always has to be regarded with caution in electron microscopy as the field is very limited, even when thousands of sections have been studied as in our investigation. Nevertheless, the negative

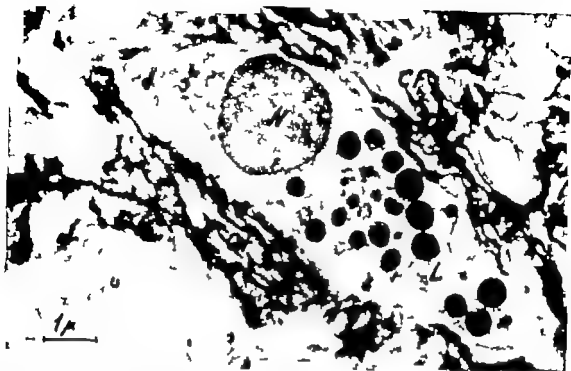


FIG 6 Electron micrograph showing the same as Fig. 5. Note the pericellular surrounding matrix with few collagen fibers (CF)  $\times 19,000$

tive finding indicates that osteoclastic bony resorption cannot be considered as the usual process in osteosclerosis. And even though osteoclastic absorption did occur it could not explain the greater part of the pathological processes, of the spread and variety of the resorption foci. On the other hand, the large number of osteocytes surrounding the foci exhibit certain morphological peculiarities which may be considered of the utmost importance to the understanding of osteosclerosis.

These abnormal osteocytes show a number of rounded cytoplasmic vesicles of different size, surrounded by a one-layered limiting membrane. These cellular organelles we have interpreted as lysosomes (Figs 5 and 6). Lysosomes, first described by Duve (1963) are attributed with a large number of enzymatic activities, so far about 15 different ones. Most of these enzymes are hydrolytic. Thus, among other activities, they are able to dissociate and dissolve protein structures, even as resistant as collagen. Lysosomes were first found in the cytoplasm of liver cells. They occur in a wide variety of cells, but have never been described in normal osteocytes. One of the first enzymes reported to be lysosomal was acid phosphatase, and its presence is still considered by many to be a good sign of lysosomal activity. In 1961 Albernaz & Covell reported the presence of this acid phosphatase in active osteosclerotic foci. Since then, it has been found by us and many others in active osteosclerotic foci. The important role of lysosomes in the resorption of bone which characterizes the initial stage of osteosclerosis is in agreement with certain findings of physiological resorption of bone (Auer



(1963) has demonstrated release of lysosomal enzymes in culture medium from bone cells undergoing resorption due to the action of the parathyroid hormone.

Moreover it has recently been emphasized by Bélanger (1965) that there might be cases of what he called osteocytic resorption without osteoclastic intervention. We believe that this is what largely happens in osteosclerosis.

It should not be inferred from our description that lysosomes are very commonly encountered in electron microscopic studies. The reason why they are not is probably that as soon as the lysosomes grow and liberate their enzymes, the cell will undergo rapid lysis. Therefore, it may be expected, statistically that a large number of sections have to be studied before coming upon cells in the very process of lysis.

What in our opinion may throw light upon the entire process of lysis in osteosclerosis is a finding in cultures of embryonic bone cells and the common knowledge that embryonic remnants abound in the fisanla ante fenestram, generally considered as the zone of its initial spread. The finding to which we are referring is that lysosomal lytic activity may be increased by oxygenating the culture perhaps only by a few per cent above the normal partial pressures of oxygen in the culture medium (Siedge, 1965).

It is well-known that 50 years ago Schwartz described marked hyperaemia as a constant sign of active osteosclerotic foci. Hyperaemia means a greater amount of oxygen to the diseased cells, and this may explain the swelling and rupture of lysosomal vesicles responsible for the lytic process. Cincea, of Bucharest, recently stated that as many as 62 theories aimed at explaining osteosclerosis by looking for the causal factor of the disease. Our opinion is quite different. We believe that the search for a cause, or even causes, will lead nowhere. We do not offer a new theory. We want to settle the problem on the cytological and cytochemical level, and we feel sure that this will prove a more fruitful way of research.

#### ACKNOWLEDGMENT

The authors acknowledge their indebtedness to Jean Causse, M.D. Béziers, France, Henrik Johansen M.D. Head of the ENT Department, Rigshospitalet, Copenhagen, and Ott Metz, M.D. Head of the ENT Department, Bispebjerg Hospital, Copenhagen, for having collaborated in procuring the histological material.

#### ZUSAMMENFASSUNG

Bei Elektronenmikroskopischen Untersuchungen osteosclerotisch im Gewebe, das unmittelbar nach Operationen dieser Krankheit fixiert ist, haben wir folgende Resultate gefunden. Erstens: Ausserhalb der beobachteten Grenzlinie des Lichtmikroskops findet man, zwischen Fokus und normalem Gewebe, „microfoci“ die man nur im Elektronenmikroskop beobachten kann. Fokus scheint bei jeder Verschmelzung dieser microfoci zu wachsen. Zweitens: Osteoclasten scheint nur

eine untergeordnete Rolle der sogenannten Resorptionsphase zu spielen. Da eben haben wir eine ausgesprochene osteocytische Resorption nachgewiesen als pericelluläre Lysis von Collagen regelmäßig gefunden sind. Im Verhältnis dazu findet man manchmal Lysosomer in den Osteocyten. Die Endothelzellen der Kapillaren scheinen normal.

## REFERENCES

- ALBARNAZ, P. L. III and COVELL, W. P. 1961: Otolocust of the larynx. A study of the lesion by histochemical procedures and fluorescence microscopy *Laryngoscope* 71: 1333.
- BÉLAÏER, L. F., SEVRA, T. and TOLAK, S. 1965: The two faces of resorption. *Calcified tissues* (Proceedings of the Third European Symposium on Calcified tissues) p. 1. Springer Verlag, Berlin.
- CAUSSE, J. 1965: Present problems in the surgery of otolaryngology. *J. Laryng.* 9: 265.
- CHEVANCE, L. G. 1960: Etude biologique dans le domaine otologique d'un cholestérol à affinité calcique. *Acta Otolaryng.* (Stockh.) 1: 46.
- 1962: *Histochimie du système spongieux*. Librairie Arnette, Paris.
- DUTCH, C. E. 1963: The lysosome concept. In *Lysosomes* (ed. A. V. S. de Renk and M. P. Cameron) p. 1. Churchill, London.
- FRAZER, R., KLOZ, G. and HALL, H. J. 1968: Microscopic diffraction electrolyses of the larynx. *Ann. Otolaryng.* (Paris) 3: 159.
- LUFT, J. H. 1961: Improvements in epoxy resin embedding. *J. Biophys. Biochem. Cytol.* 9: 409.
- REIDON, J. L., and SMITH, C. A. 1968: The ultrastructure of normal and otolaryngic human larynx. *Laryngoscope*, 78: 93.
- SLEDAK, C. B. 1965: Lysosomes and cartilage resorption in vitro. *Calcified tissues* (Proceedings of the Third European Symposium on Calcified tissues) p. 52. Springer Verlag, Berlin.
- VAN, G. 1965: Acid hydrolysis, lysosomes and bone resorption induced by parathyroid hormone. *Calcified tissues* (Proceedings of the Third European Symposium on Calcified tissues) p. 36. Springer Verlag, Berlin.

L. G. Chevance, M.D.  
 Lab. d'Otologie, Faculté de  
 Médecine, Paris, France  
 Dr. Balsem Jørgensen, M.D. The ENT  
 Clinic, Rigshospitalet, Copenhagen  
 Denmark

Received September 9, 1968

## GLYCOGEN IN THE INNER EAR AFTER ACOUSTIC STIMULATION

### *A light and electron microscopic study*

D. ISHII, T. TAKAHASHI and J. BALOGH

*From the Massachusetts Eye and Ear Infirmary, Massachusetts General Hospital  
and Department of Otolaryngology and Pathology, Harvard Medical School,  
Boston, Mass., U.S.A.*

Glycogen was localized in the inner ear of various species with the light and electron microscopic techniques. Several techniques of tissue fixation and embedding were tested. Light microscopic demonstration of glycogen was consistent and optimal in glutaraldehyde-fixed and Epon-embedded specimens stained with the periodic acid-Schiff reaction. With this standard method, well-defined intracytoplasmic glycogen granules were red (purple) with the periodic acid-thiosemicarbazide method for electron microscopy demonstration of glycogen. Numerous lamellar electron-dense particles were seen in the same cell regions in which the PAS-positive granules were observed. These electron-dense granules were digestible with  $\alpha$ -amylase.

The distribution pattern showed marked species differences. In guinea pigs, glycogen granules were limited to the outer hair cells of the cochlea with a decrease basally. In mice, most of the glycogen granules were seen in Deiters cells and less in the outer hair cells. In cats, no glycogen could be demonstrated. In none of these species was glycogen observed in the vestibular sensory and supporting cells.

After exposing guinea pigs to 110 dB white noise for 30 minutes, there was a quantitative decrease of glycogen. Three hours after acoustic stimulation, numerous small granules were detectable and in 12 to 24 hours these aggregated to form bigger granules. Finally 24 hours after the exposure to noise the distribution pattern of glycogen reverted to normal. These changes following acoustic stimulation may be due to glycogenolysis and subsequent glycogenesis, suggesting that glycogen serves as an energy source in the hair cells.

Glycogen,  $(C_6H_{10}O_5)_n$ , a polysaccharide made up from glucose units and distributed through the cytoplasm as a source of energy via glycogenolysis. Considering the blood supply of the organ of Corti, anaerobic glycolysis may have an important role in its metabolism with glycogen as the main source of energy. Although several investigators have studied the

This study supported by research grant NS 01123-05 from the National Institute of Neurological Disorders and Blindness, National Institutes of Health of the United States Public Health Service.

TABLE 1 *Review of light microscopic studies*

Species	Authors	Localization				Remarks
		Inner hair cells	Outer hair cells	Dieters cells	Hansen's cells	
Guinea pig	Zorzoif 1951	++	++			
	Vinnikov & Tlova 1957	-	++			surface (flat) specimen
	Flatz, 1958	-	++			
	Falbe-Hansen & Thomsen, 1963	-	++			
	Present authors	-	++			
Mouse	Present authors	-	±	++	±	
Bat (hibernating)	Ploix & Perlman, 1953	+	-	++		freeze-dried specimen
Rat	Bélanger 1956	-	++	+		
Rabbit	Vinnikov & Tlova, 1957	++	++	++	++	surface (flat) specimen
Cat	Vinnikov & Tlova, 1957	-	+	++	++	surface (flat) specimen
	Falbe-Hansen & Thomsen 1963	-	-	-	-	
	Present authors	-	-	-	-	

- glycogen not demonstrable    ± little or no glycogen    + moderate amount of glycogen  
 ++ rich in glycogen    glycogen decreasing basallywards.

distribution of glycogen in normal and acoustically traumatized animals, there appears to be a great discrepancy among the published results (see Table 1)

Therefore we have compared the results of various histochemical techniques and identified glycogen with the light and electron microscope in normal animals. In order to clarify the controversy about the effects of acoustic stimulation on glycogen in the organ of Corti, we have also exposed guinea pigs to white noise. In this publication we report the results of these studies.

#### MATERIAL AND METHODS

##### *Light microscopy*

Ten young albino guinea pigs, two young cats and two young albino mice were sacrificed under light anesthesia with ether or pentobarbital sodium. Their temporal bones were removed, trimmed, stapedectomized and fixed with Lillie's picric acid alcohol (4 C) for 24 hours or in 2.5% buffered glutaraldehyde (4 C) for 20 minutes. They were then dehydrated with alcohol and embedded in Epon mixture.



Fig. 1 Glycogen in the organ of Corti of normal guinea pigs. (a) Apical turn, (b) 3rd turn, (c) 2nd turn, (d) basal turn. The amount of glycogen decreases towards the basal turn. Glutaraldehyde-fixed, Epon-embedded tissues stained with the periodic acid-Schiff (PAS) reaction. (e) Freeze-dried, Epon-embedded tissue; PAS reaction. Beside the granular appearance there is diffuse cytoplasmic stain. (f) Glycogen granules appear more distinct in Seligman's method. All photomicrographs were taken at the same magnification.



Two temporal bones of guinea pigs were dropped in isopentane chilled in liquid nitrogen, followed by freeze-drying with a Speedvac Pearse tissue dryer Model 1 fixed overnight at 85 C in the vapor phase of formaldehyde and directly embedded in Epon mixture.

Epon blocks were cut at 3  $\mu$  with glass knives using a Porter Blum ultramicrotome. Sections were picked up with a drop of water and slightly warmed to stick firmly on cover slips. Glycogen was demonstrated with the periodic acid Schiff (PAS) reaction (MacManus, 1948) or with Seligman's periodic acid-thiosemicarbazide technique (Seligman *et al* 1963). For both methods sections were oxidized for 90 minutes in a 0.5% solution of periodic acid and kept for another 90 minutes in the respective reagent solution. In the case of Seligman's method, the sections were placed in a 1% buffered osmium tetroxide solution for 30 minutes. In some instances glycogen was digested with a 1%  $\alpha$ -amylase solution (buffered to pH 6.0) prior to staining. All sections were dehydrated and mounted with synthetic mounting media.

### Electron microscopy

Two temporal bones of guinea pigs were processed for electron microscopic studies. Tissues were fixed and embedded as for light microscopy and ultrathin sections were cut with a diamond knife on an LKB ultratome. Section picked up on gold grids were treated with 0.5% periodic acid solution for 45 minutes, then for 45 minutes in a 1% thiosemicarbazide solution (the solvent was 23% acetic acid in 70% alcohol) afterwards kept in a 1% buffered osmium tetroxide for 30 minutes and finally stained with uranyl acetate and lead citrate. For enzymatic digestion of glycogen, sections were incubated (37 C) with a 1% buffered  $\alpha$ -amylase solution for 45 minutes prior to staining (Rosa & Johnson, 1967). Specimens were then examined and photographed under a Siemens Elmiskop 1.

### Acoustic trauma

Fifteen young albino guinea pigs were exposed for 30 minutes to 110 dB white noise by reference to 0.0002 dyne  $\text{cm}^2$  in a free field. Animals in groups of three were sacrificed 0, 3, 6, 12 and 24 hours after the noise exposure and cochleas processed for light microscopy.

## RESULTS

### Normal animals

#### Light microscopy

Tissues were better preserved with 2.5% glutaraldehyde fixation than with 10% picric acid alcohol which produced marked shrinkage of the hair cells. In glutaraldehyde fixed cochleas, glycogen granules were stained magenta red with the PAS reaction (Fig. 1a-d). In tissues digested with  $\alpha$ -amylase before staining, the reaction was not completely negative but





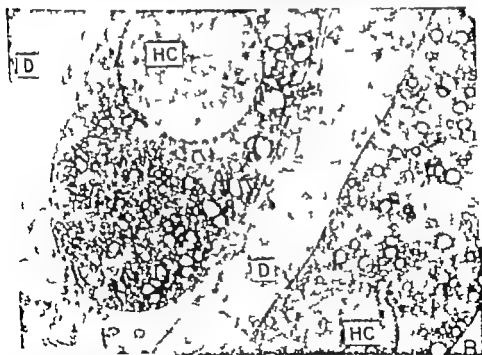


FIG. 3. Electron micrographs of glycoproteins of Corti stained with osmium tetroxide for glycogen. *A* Two outer hair cells (HC) contain numerous electron-dense glycogen granules that appear black. *B* Specimen digested with  $\alpha$ -amylase before osmium reaction. The dark area of the microcytoplasmic space corresponds to that of the glycogen granules. The nuclei of two outer hair cells (HC) and the cytoplasm of supporting cells (D) did not contain digestible material.  $\times 3000$ .



FIG. 2. Striking species differences are evident there is abundant glycogen in the supporting cells of mice (A) but not demonstrable in cats (B). Glutaraldehyde-fixed, Epon-embedded, PAS-stained tissues from upper turn of cochlea  $\times 330$ .

became much weaker. In comparison freeze-dried tissues showed considerable variation in tissue preservation frequently the hair cells appeared shrunken. However the PAS reaction was strongly positive throughout the cytoplasm of the shriveled outer hair cells, perhaps due to the concentration of glycogen. Better preserved hair cells showed a fine reticular pattern of PAS-positive material (Fig. 1c). There was some difference in the distribution of glycogen using the PAS or Seligman's methods; the latter showed more numerous and finer granules in the cytoplasm of the outer hair cells (Fig. 1f).

Marked species differences were noted. In guinea pigs the outer hair cells of the apical area were very rich in glycogen but the reaction gradually decreased towards the base until no PAS-positive material was demonstrable in the lower half of the basal turn (Fig. 1a-d). The inner hair cells and the vestibular sensory cells were almost completely without glycogen granules. In mice glycogen was abundant in the supporting cells, especially in Deiters cells, whereas the outer hair cells contained only a few granules (Fig. 2A). There were no appreciable differences between various turns of the cochlea as in guinea pigs. In cats glycogen was not demonstrable with either histochemical method (Fig. 2B).

### *Electron microscopy*

Ultrathin sections incubated with Seligman's method contained numerous well defined black granules throughout the cytoplasm of the outer hair cells (Fig. 3A). The granules were roughly isodiametric and appeared larger than unstained glycogen particles. The great majority of these granules were missing in specimens digested with  $\alpha$ -amylase. On the other hand, numerous round or oval clear spaces were seen in such sections. The distribution of these spaces corresponded to that of the glycogen granules (Fig. 3B). Supporting cells, nerve endings and inner hair cells showed only sparse granules.

wards the size of the granules decreased but they were more numerous than normal. Between 12 and 24 hours after exposure, the granules aggregated and appeared bigger although less numerous. Finally 24 hours after the noise exposure, the glycogen granules in the outer hair cells appeared normal in most of the cases (Fig. 4 1-D). Despite this general tendency however no appreciable changes could be detected in a few animals.

## DISCUSSION

Several histochemical methods have been recommended for the light and electron microscopic localization of glycogen. The specificity of all these methods ultimately depends on the enzymatic digestion of the demonstrated substance. For light microscopy the PAS reaction controlled by enzymatic digestion is one of the most commonly used techniques, even though it has some disadvantages. For instance enzymatic digestion may be incomplete in thick histological sections, or glycogen granules may be resistant to digestion in fixed tissues. Another problem is due to the water solubility of glycogen, which may partly elude demonstration with aqueous reagents. Of course there remains a possibility that protein-bound glycogen (deamoglycogen) is not demonstrated with these histochemical methods (Kugler & Wilkinson, 1960). Watson (1958) used lead citrate staining for electron microscopic localization of glycogen after glutaraldehyde and/or osmium tetroxide fixation with this method glycogen appears as approximately biulametric particles ( $\beta$ -particles) or their aggregates ( $\alpha$ -particles). Seligman and co-workers in 1963 established a new method using periodic acid oxidation and a group of chemicals such as thiocarbonylhydrazide or thiosemicarbazide followed with post-osmication, which theoretically seems more specific than lead citrate staining. Rosa & Johnson (1967) have recently been able to digest glycogen particles in Epon-embedded ultrathin sections, indicating that the specificity of the electron microscopic observations can be checked. At the present, this seems to be the most reliable electron microscopic technique for the visualization of glycogen because the digestion pattern is rather distinct and consistent.

The inner ear presents special technical problems for the histochemical demonstration of glycogen. At the present, bones cannot be decalcified without loss of glycogen (Trott *et al.* 1982) therefore the cochlea must be sectioned without decalcification. Another though less formidable, obstacle is proper fixation. It is true that alcoholic fixatives will preserve glycogen for light microscopy but they produce several artifacts such as cell shrinkage intracellular "polarization" ("Alkoholflucht") of glycogen etc. Both phenomena are quite striking in the organ of Corti, especially in the outer hair cells. This is understandable because the hair cells are directly exposed to fixatives. We obtained satisfactory fixation and preservation of glycogen with short-term fixation in cold glutaraldehyde followed by embedding in

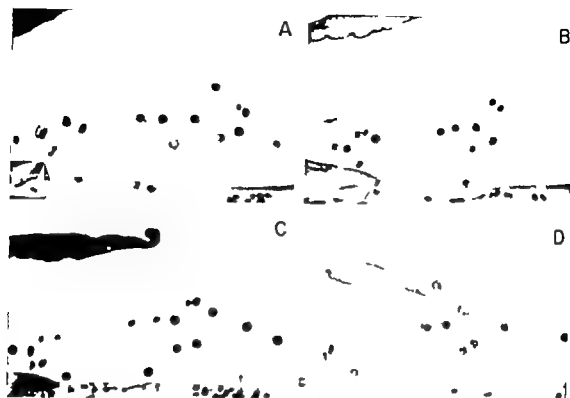


FIG. 4 Effect of acoustic stimulation on glycogen content of outer hair cell of guinea pigs. (A) Normal intracytoplasmic glycogen is finely granular. (B) Three hours after exposure to white noise (110 dB for 30 min) Glycogen content is decreased. (C) Six hours after exposure to white noise Glycogen granules are very small but quite numerous. (D) Twelve hours after exposure to white noise. Glycogen granules are larger and less numerous than normal. All photographs are from the organ of Corti at the low 2nd turn. Glutaraldehyde fixed, Epon-embedded tissues with the PAS reaction.  $\times 330$

### Acoustically Traumatized Animals

The findings in this group were variable, even though care was taken to eliminate possible differences in tissue processing. To further minimize variations, observations were made in a standard area, namely 7.0–9.5 mm above the basal end of the cochlea. This specific area can be easily identified, because the secondary osseous spiral lamina is still present but the Boettcher cells are absent<sup>1</sup>. Although maximal damage could be expected in this area (Nakamura, 1964) the outer hair cells were missing only in one among 30 cochleas. Generally, the number of glycogen granules in the examined area decreased up to 3 hours after the acoustic trauma after

When the middle temporal bones of 12 guinea pigs of different ages and that of the aged Boettcher cell and the secondary osseous spiral lamina extended from the basal end up to 7.43 (standard deviation 0.47) mm and to 9.30 (0.34) mm, respectively, the total length of the organ of Corti was 18.33 (0.69) mm.

According to Nakamura (1964) acoustic trauma with white noise in the middle range in the 9.6–10.3 mm range of guinea pig cochlea. Since the total length of the organ of Corti in the middle range was  $19.7 \pm 2.04$  mm, our observations in the 7.43–9.30 mm area (of short organ of Corti) are comparable.

wards the size of the granules decreased but they were more numerous than normal. Between 12 and 24 hours after exposure, the granules aggregated and appeared bigger although less numerous. Finally 24 hours after the noise exposure the glycogen granules in the outer hair cells appeared normal in most of the cases (Fig. 4 A-D). Despite this general tendency however no appreciable changes could be detected in a few animals.

## DISCUSSION

Several histochemical methods have been recommended for the light and electron microscopic localization of glycogen. The specificity of all these methods ultimately depends on the enzymatic digestion of the demonstrated substance. For light microscopy the PAS reaction controlled by enzymatic digestion is one of the most commonly used techniques, even though it has some disadvantages. For instance enzymatic digestion may be incomplete in thick histological sections, or glycogen granules may be resistant to digestion in fixed tissues. Another problem is due to the water solubility of glycogen, which may partly elude demonstration with aqueous reagents. Of course there remains a possibility that protein-bound glycogen (desmoglycogen) is not demonstrated with these histochemical methods (Kugler & Wilkinson, 1960). Watson (1958) used lead citrate staining for electron microscopic localization of glycogen after glutaraldehyde and/or osmium tetroxide fixation with this method glycogen appears as approximately isodiametric particles ( $\beta$ -particles) or their aggregates ( $\alpha$ -particles). Seligman and co-workers in 1965 established a new method using periodic acid oxidation and a group of chemicals such as thiocarbonylhydrazide or thiosemicarbazide followed with post-osmication, which theoretically seems more specific than lead citrate staining. Ross & Johnson (1967) have recently been able to digest glycogen particles in Epon-embedded ultrathin sections, indicating that the specificity of the electron microscopic observations can be checked. At the present, this seems to be the most reliable electron microscopic technique for the visualization of glycogen because the "digestion pattern" is rather distinct and consistent.

The inner ear presents special technical problems for the histochemical demonstration of glycogen. At the present, bones cannot be decalcified without loss of glycogen (Trotti *et al.* 1962) therefore the cochlea must be sectioned without decalcification. Another though less formidable, obstacle is proper fixation. It is true that alcoholic fixatives will preserve glycogen for light microscopy but they produce several artifacts such as cell shrinkage intracellular "polarization" (Alkoholflucht) of glycogen etc. Both phenomena are quite striking in the organ of Corti, especially in the outer hair cells: this is understandable because the hair cells are directly exposed to fixatives. We obtained satisfactory fixation and preservation of glycogen with short-term fixation in cold glutaraldehyde followed by embedding in

**Epon** This technique was adapted after comparison with freeze-dried specimens in which the loss of glycogen was supposedly minimal. A definite answer cannot be given for the incomplete digestion of glycogen with  $\alpha$ -amylase. We attributed this to the slow penetration of the enzyme into the relatively thick sections embedded in Epon. Evidence for this possibility was provided by the electron microscope which revealed complete digestion of glycogen particles in ultrathin sections. Apparently these technical problems explain in part the contradictory reports on the distribution of glycogen in the cochlea. Species differences account for other discrepancies (see also Table 1).

Our observations in guinea pigs and cats confirm the results of Finzi (1958) and Falbe Hansen & Thomsen (1963). While no data are available on mice to compare with our findings, it is interesting to note that a similar distribution exists in bats (Plotz & Perlman 1965). These marked species differences caution us not to generalize about the metabolic role of glycogen in the cochlea.

The presence of glycogen particles in the organ of Corti of guinea pigs has been noted by several electron microscopists, but these observers have not provided histochemical evidence. It was first Vosteen (1964) who specifically demonstrated glycogen in the organ of Corti. He used carmine red as a contrast stain for glycogen and found the cytoplasm of the outer hair cells densely packed with carmine positive particles. Moderate amounts were noticed also in the nerve endings and in the inner hair cells. Unfortunately there are no electron microscopic data on the correlation between carmine-positivity and digestion with  $\alpha$ -amylase. Even with Seligman's method we found undigestible stained particles. Therefore, histochemical reactions for glycogen cannot be considered absolutely specific unless confirmed by enzymatic digestion. Using this as a relatively reliable technique we demonstrated glycogen exclusively in the outer hair cells of the guinea pig.

The findings in acoustically traumatized guinea pigs do not permit definite conclusions about the possible role of glycogen in the metabolism of acoustically traumatized hair cells. Nevertheless, it is reasonable to assume that the transient decrease in the number of glycogen granules followed by an increase in smaller granules may be due to glycogenolysis and consecutive glycogenesis. This would imply the enzymatic release of chemical energy after acoustic stimulation and restoration of the depleted glycogen stores by enzymatic process. However the marked species differences in the distribution of glycogen indicate that this process is not necessarily operative in the cochlea of all mammals.

#### ACKNOWLEDGMENT

We are indebted to Dr Nelson Y. Kiang and Miss Diane Demont for assistance with the acoustic stimulation experiments.

## ZUSAMMENFASSUNG

Glykogen wurde mit dem Licht und Elektronenmikroskop im Innenohr verschiedener Säugetiere dargestellt. Dabei wurden mehrere Fixations- und Einbettungsverfahren geprüft. Der Lichtmikroskopische Nachweis von Glykogen war übereinstimmend und optimal mit der Perjodäure-Schiff'schen (PAS) Reaktion in Präparaten, die in Glutaraldehyd fixiert und in Epon eingebettet wurden. Mit dieser Standardmethode farbten sich die wohlbekannten cytoplasmatischen Glykोग्रanula rot bis violett. Mit der Perjodäure-Thiosemicarbazid-Methode von Seligman zum elektronenoptischen Nachweis von Glykogen wurden in den gleichen Zellteilen wie die PAS-positiven Granula zahlreiche dunkle isodiametrische Partikel beobachtet. Diese elektronendichten Granula konnten mit  $\alpha$ -Amylase verestert werden.

Das Verteilungsmuster wies grosse Unterschiede zwischen verschiedenen Tierarten auf. In Meerschweinchen waren die Glykogenkörnchen auf die äusseren Haarzellen begrenzt und abnahmen baswärts ab. In Mäusen wurde Glykogen hauptsächlich in den Deiterschen Zellen beobachtet und weniger in den äusseren Haarzellen. In Katzen konnte kein Glykogen nachgewiesen werden. In keiner der untersuchten Tierarten war Glykogen in den vestibulären Sinnes- und Motorzellen nachweisbar.

Nach Belastung mit 110 dB white noise (30 Minuten) nahmen die Glykogenkörnchen ab. Nach drei Stunden konnte man zahlreiche kleine Granula darstellen, die sich 12 bis 24 Stunden nach der Belastung zu grösseren Körnchen anordneten. Nach 24 Stunden war das Verteilungsmuster von Glykogen wieder normal. Die Veränderungen nach dem akustischen Reiz können mit einer Glykolyse und nachfolgendem Glykogenaufbau erklärt werden, und weisen darauf hin, dass Glykogen in den inneren Haarzellen von Meerschweinchen eine muskuläre Energiequelle darstellt.

## REFERENCES

- BILHAM, L. F. 1968 Observations on the development, structure and composition of the cochlea of the rat. *J. Otol. & Otorrh.* 10: 60.
- FRANKE, H. and T. OSMAN, E. 1963 Histochemical studies on glycogen in the cochlea of the normal guinea pig. *Acta Otolaryng. Stockh.* 56: 129.
- FRANKE, H. 1964 Reticular Histochemie (Organ der Corti und des Cellula sensoria). *Arch. Hist. Ital. Otol.* 69: 817.
- FRANKE, H. and WILKINSON, W. J. C. 1966 Glycogen fractions and their role in the histochemical detection of glycogen. *J. Histochem. Cytochem.* 15: 193.
- MULLER, C. J. F. 1918 Histological and histochemical work of peroxidic acid. *Stain Techn.* 23: 99.
- NAKANO, S. 1961 Some of the basic problems in noise trauma. The 6th Japan Otorhinolaryngology Society Meeting, T. Kashiwa, Japan.
- PIETZ, E. and STALIN, C. H. H. 1955 Histochemical study of the cochlea. *Laryngoscope* 65: 291.
- ROSE, P. and JOHNSON, P. B. 1967 Enzymatic digestion in the cytochemical demonstration of glycogen. *J. Histochem. Cytochem.* 15: 14.
- SELIGMAN, A. M. H., KEN, J. S., W. MARGALIT, H. DIMONOVSKY, H. and KATZ, L. 1963 Histochemical demonstration of some oxidized macromolecules with thiocarbonylhydrazide or thiosemicarbazide and some in vitro tests. *J. Histochem. Cytochem.* 12: 622.

- TROTT J R, GORIKSTEIN, S. L., and PERKOFF M. D. 1962 A chemical and histochemical investigation of glycogen in rat liver and palate following treatment with various fixatives and ethylenediamine tetraacetic acid. *J Histochem Cytochem* 10 245.
- VIMNIKOV YA A., and TIROVA, L. K., 1957 Distribution of glycogen in the organ of Corti with and without sound stimulation. *Dokl Ak d. Nauk SSSR* (Moskva) 116 930 (In Russian.)
- VOSTREK K. H. 1964 Elektronenmikroskopische Untersuchungen über die Verteilung von Glykogen im Ductus cochlearis bei Meerschweinchen. *Pract Ot rhinolaryng.* (Basl) 26 400
- WATSON M L., 1958: Staining of tissue sections for electron microscopy with heavy metals. II Application of solutions containing lead and barium. *J Biophys. Biochem. Cytol* 4 737
- ZONZOLI, G. C., 1954: Recherches histochemiques sur l'oreille interne (des cobayes) *C R. Assoc. Anatom* 41 785.

K Balogh M.D. Massachusetts  
Eye and Ear Infirmary  
243 Charles Street  
Boston Mass 02114 U.S.A

Received August 24 1968



## SOME MORPHOLOGICAL ASPECTS OF SOUND PERCEPTION IN BIRDS

V. JÄNDER, P.-G. LUNDQUIST and J. WERSÄLL

From the Department of Otolaryngology Karolinska Sjukhuset, and the King Gustaf V Research Institute Stockholm Sweden

The fine structure of the cochlear duct of the cock (*Gallus domesticus*) was studied by light and electron microscopy. The morphological findings confirmed known physiological facts.

Because of the obvious difficulties associated with studies of the morphology and neurophysiology of the human ear most of the investigations on which our understanding of the function of the peripheral hearing organ is based on other mammals. However even studies on mammals are not enough to provide a clear picture of the conversion of mechanical energy from the mechanoreceptors to nerve impulses, and comparative morphology and physiologic research on a wide basis would therefore seem to be essential.

As a link in our investigations of the morphology and function of the mechanoreceptors an attempt has been made in the present study to throw light on the fine structure of the organ of Corti in the cock and to correlate these observations with the known physiologic facts.

The architectonics of the organ of Corti as it is seen under the microscope is familiar from a series of studies by Hasse (1867), Retzius (1884), Held (1926), de Burlet (1934), Stresemann (1934), Kolmer (1928) and others. An excellent survey of the earlier work in this field has been published by Frey (1952). The fine structure of the cochlea in birds has been described by Cordier (1964) and Vinnikov *et al.* (1965).

### *Light Microscopical Technique*

A number of young adult cocks were perfused through the aorta with Heidenhain-Susa solution and the heads subsequently fixed in the same solution, decalcified and bedded in celloidin. The imbedded specimens were then cut in a Leitz cellidion specimen microtome into serial sections with a mean thickness of about  $12\ \mu$ . The sectioned specimens were stained with haematoxylin and eosin solution and examined with a Zeiss photomicroscope.

### *Electron Microscopical Technique*

Eighteen young adult cocks were anaesthetized deeply with Nembutal and decapitated. The temporal bone was dissected and the columella lifted from the oval window. The vestibulum and cochlea were carefully perfused with osmium tetroxide solution (Rhodin, 1954) and the specimen removed and fixed in the same solution. During the subsequent dehydration the various parts of the labyrinth were dissected and imbedded separately in Epon 812 (Luft 1961).

For pilot studies about 1  $\mu$  thick sections were cut with an LKB ultratome and stained with toluidine blue solution. After careful orientation of the specimen ultra thin sections were then cut with an LKB ultratome stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I. Gevaert Scientia 22 D 50 plates were used for photography at an enlargement of between 1000 and 40 000 times.

### *Light Microscopic Examination*

The light microscopic picture of the inner ear of birds has been described by a large number of research workers. It will suffice here therefore to give a short summary of the structure of the membranous labyrinth as a basis for the subsequent detailed description of its fine structure.

#### *Development*

The basilar papilla or the organ of Corti of birds is developed from the pars inferior of the membranous labyrinth. As in the turtle in the early stages of development the sacculus is still slightly widened to form a small recess, containing the lagena and lacinia. The lagena is an area of sensory epithelium covered by otoliths, whereas the lacinia hair cells are in contact with a tectorial membrane.

In birds and reptiles this recess is further developed into an elongated tube which, suspended in a cartilaginous frame separates the surrounding perilymphatic fluid into two portions, the scala tympani and the scala vestibuli. These are equivalent to those of the higher vertebrates. This membranous tube forms the slightly curved cochlear duct of the bird which contains the organ of Corti, resting on a basement membrane separating the cochlear duct from the scala tympani (Fig. 1). The extreme peripheral part of the tube contains the macula lagena, with its otolith-covered sensory cells (Fig. 2).

#### *The cochlear duct*

The cochlear duct is separated from the scala vestibuli by the tegmentum vasculosum (Figs. 1-3). This is a membrane consisting of a loose connective tissue, containing a dense capillary network. The richly folded surface which faces the endolymph of the cochlear duct, is covered by an epithelium consisting of light cells and intensely staining dark cells (Fig. 3). These two cell types were described by Stresemann (1934) and their presumed secre-

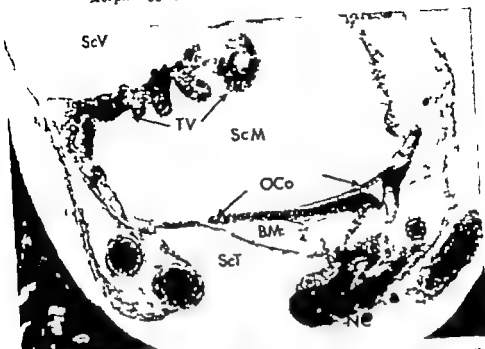


Fig. 1. Light microscopic survey demonstrating the organ of Corti (OCo) standing on the basilar membrane (BM) suspended in articular frame. The anterior semicircular canal (SC) on right. The scala vestibuli (ScV) is separated from scala media (ScM) by specialized membrane, the tegmen tectum (TV). The scala tympani (ScT) is below the basilar membrane at the bottom of the picture.

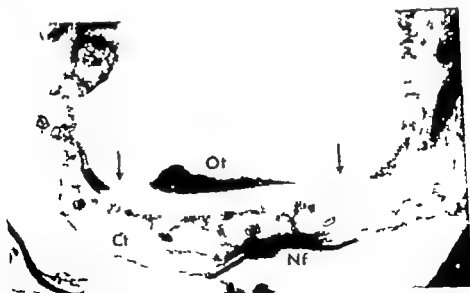


Fig. 2. Macula lagena (arrows) with otolith (Ot). The nerve fibers (NF) are seen penetrating the connective tissue (Ct).

### *Electron Microscopical Technique*

Eighteen young adult cocks were anaesthetized deeply with Nembutal and decapitated. The temporal bone was dissected and the collumella lifted from the oval window. The vestibulum and cochlea were carefully perfused with osmium tetroxide solution (Rhodin, 1954) and the specimen removed and fixed in the same solution. During the subsequent dehydration the various parts of the labyrinth were dissected and imbedded separately in Epon 812 (Luft 1961).

For pilot studies about 1  $\mu$  thick sections were cut with an LKB ultratome and stained with toluidine blue solution. After careful orientation of the specimen ultra thin sections were then cut with an LKB ultratome, stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop L. Gevaert Scientia 22 D 50 plates were used for photography at an enlargement of between 1000 and 40 000 times.

### *Light Microscopic Examination*

The light microscopic picture of the inner ear of birds has been described by a large number of research workers. It will suffice here therefore to give a short summary of the structure of the membranous labyrinth as a basis for the subsequent detailed description of its fine structure.

### *Development*

The basilar papilla or the organ of Corti of birds is developed from the pars inferior of the membranous labyrinth. As in the turtle in the early stages of development the sacculus is still slightly widened to form a small recess, containing the lagena and lacinia. The lagena is an area of sensory epithelium, covered by otoliths, whereas the lacinia hair cells are in contact with a tectorial membrane.

In birds and reptiles this recess is further developed into an elongated tube which, suspended in a cartilaginous frame, separates the surrounding perilymphatic fluid into two portions, the scala tympani and the scala vestibuli. These are equivalent to those of the higher vertebrates. This membranous tube forms the slightly curved cochlear duct of the bird which contains the organ of Corti, resting on a basement membrane separating the cochlear duct from the scala tympani (Fig. 1). The extreme peripheral part of the tube contains the macula lagena with its otolith-covered sensory cells (Fig. 2).

### *The cochlear duct*

The cochlear duct is separated from the scala vestibuli by the tegmentum vasculosum (Figs. 1-3). This is a membrane consisting of a loose connective tissue containing a dense capillary network. The richly folded surface, which faces the endolymph of the cochlear duct is covered by an epithelium consisting of light cells and intensely staining dark cells (Fig. 3). These two cell types were described by Stressemann (1934) and their presumed secre-



### *Electron Microscopical Technique*

Eighteen young adult cocks were anaesthetized deeply with Nembutal and decapitated. The temporal bone was dissected and the collumella lifted from the oval window. The vestibulum and cochlea were carefully perfused with osmium tetroxide solution (Rhodin 1954) and the specimen removed and fixed in the same solution. During the subsequent dehydration the various parts of the labyrinth were dissected and imbedded separately in Epon 812 (Luft 1961).

For pilot studies about 1  $\mu$  thick sections were cut with an LKB ultratome and stained with toluidine blue solution. After careful orientation of the specimen ultra thin sections were then cut with an LKB ultratome, stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I. Gevaert Scientia 23 D 50 plates were used for photography at an enlargement of between 1000 and 40 000 times.

### *Light Microscopic Examination*

The light microscopic picture of the inner ear of birds has been described by a large number of research workers. It will suffice here therefore to give a short summary of the structure of the membranous labyrinth as a basis for the subsequent detailed description of its fine structure.

### *Development*

The basilar papilla or the organ of Corti of birds is developed from the pars inferior of the membranous labyrinth. As in the turtle in the early stages of development the sacculus is still slightly widened to form a small recess, containing the lagena and lacina. The lagena is an area of sensory epithelium covered by otoliths, whereas the lacina hair cells are in contact with a tectorial membrane.

In birds and reptiles this recess is further developed into an elongated tube which, suspended in a cartilaginous frame separates the surrounding perilymphatic fluid into two portions, the scala tympani and the scala vestibuli. These are equivalent to those of the higher vertebrates. This membranous tube forms the slightly curved cochlear duct of the bird which contains the organ of Corti resting on a basement membrane separating the cochlear duct from the scala tympani (Fig. 1). The extreme peripheral part of the tube contains the macula lagena, with its otolith-covered sensory cells (Fig. 2).

### *The cochlear duct*

The cochlear duct is separated from the scala vestibuli by the tegmentum vasculosum (Figs. 1-3). This is a membrane consisting of a loose connective tissue, containing a dense capillary network. The richly folded surface, which faces the endolymph of the cochlear duct is covered by an epithelium consisting of light cells and intensely staining dark cells (Fig. 3). These two cell types were described by Stresemann (1934) and their presumed secre-

tory function has been studied by among others, Dohleman and co-workers (1959)

Most of the lower tympanal wall of the cochlear duct is occupied by the basilar papilla, a wide vaulted epithelial ridge corresponding to the organ of Corti in mammals. A minor part of the papilla rests on the cartilage capsule while most of it extends over the basilar membrane, which separates the cochlear duct from the scala tympani, and is stretched between the lip-shaped cartilage plates projecting from the anterior and posterior wall in the cartilage capsule (Figs. 1-4)

The basilar membrane can be divided into two regions—an anterior wider zone and a posterior narrower zone. The former on which most of the basilar papilla rests, is lenticular in section and consists of several layers. Facing the perilymph in the scala tympani is a layer of low cuboidal connective tissue cells, which cover the true basilar membrane. This is composed of fairly regular parallel fibrils, imbedded, in the fixed specimen, in a fine filamentous matrix. On this membrane rests a layer of irregular connective tissue cells, which forms an extremely loose stratum, poor in cells, and comprising the greater part of the lenticular membrane. This layer is divided in its turn from the true epithelium by a thin basal membrane on which the supporting cells of the basilar papilla rest (Fig. 4)

#### *Basilar papilla (organ of Corti)*

The supporting cells of the basilar papilla are elongated, slightly irregular cylindrical elements, which rest on the basilar membrane and extend up to the surface of the basilar papilla, where they comprise the sensory cells. Under the microscope the cells are light and have little structure. The basilar papilla is surrounded peripherally by the anterior and posterior hyaline cells (Figs. 1 and 4)

From the anterior ones a fine fibrillar membrane with sometimes hyaline appearance, the tectorial membrane, projects over the sensory epithelium. The membrane surface facing the sensory epithelium is intimately connected with this through projecting strands that connect the tectorial membrane with the upper side of the supporting cells, so that the membrane forms domes over the individual sensory cells (Fig. 4)

#### *Sensory cells*

The sensory cells are rod-shaped ranging in length from 8 to  $24\ \mu$ . The section is hexagonal, about  $6\ \mu$  across. The sensory cells are longest at the top of the curved surface of the sensory epithelium, and diminish in both directions. Each cell gives off a bundle of sensory hairs, which are intimately enclosed in the lower surface of the tectorial membrane

#### *Vocal fibers*

The distal myelinated axons of the cochlear ganglion cells located in the anterior cartilage lamina innervate the sensory cells of the basilar papilla.



FIG. 3 Tegmentum vasculatum with light cells (LC) and dark cells (DC). Scula vestibuli (ScV), scula media (ScM). Note large capillaries (arrows).

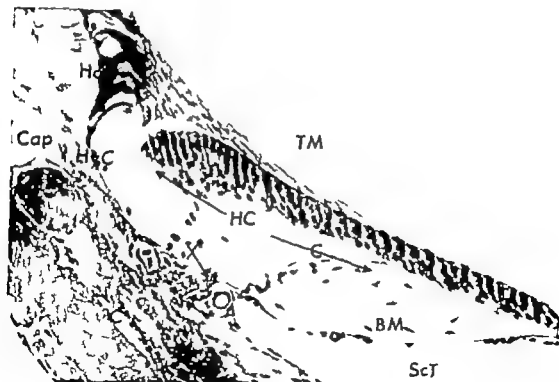


FIG. 4 Detail of the bulbular papilla region of Cortex with the tectal membrane (TM) being uppeled in a tent-like fashion from the hindgem cell (HC). The hindgem cell (HGC) below the bulbular membrane (BM). A hindgem cell can be seen penetrating the scutellum (SC) and lying beneath the myelinated sheath (arrow). A large capillary is seen (Cap). The scula tympani (ST) is at the bottom of the picture.





FIG. 6. Hair cell top with cuticle (Ca) and sensory hairs (SH). There is a completely developed kinocilium (K) connected to the basal body (BB) in the part of the cell free of cuticle. In the posterior part of the basal body a basal foot (BF) is regularly present. 12,800.

On leaving the cartilage the nerve fibers lose their myeline sheath spread fanwise along the basilar membrane and turn upwards under the sensory cells, at the bottom of which they form nerve endings.

It has been proposed that small unmyelinated nerve fibers innervate the secretory cells of the tegmentum vasculosum (Kolmer 1928).

### *Electron Microscopy*

#### *Sensory hairs and cuticle*

The top of the hair cell is partly covered by a dense substance forming the cuticle and located just within the plasma membrane of the flat top of the cell. About 100 sensory hairs protrude from the cuticle (Fig. 5). The posterior part of the hair-cell top has no cuticle in this region there is a "basal body" or centriole from which protrudes a kinocilium of varying length and development (Fig. 6). The sensory hair which is about  $0.2 \mu$  in diameter is composed of a central, fine fibrillar core this is slightly denser at the periphery where it is surrounded by a plasma membrane of the cell (Fig. 7). The axial core of the hairs form a rootlet, which continues into the cuticle. The development of the kinocilium is most advanced in the



FIG. 5. The sensory cell is rod shaped, with dense region in the center (Cu) lying only the posterior part free. A bundle of sensory hairs (SH) are seen penetrating the cuticle. There is a kinocilium, which has a striated appearance (KC). The top of the hairs are in close contact with the tectorial membrane (TM). The nucleus (Na) is located about  $5 \mu$  from the top of the hair. The secretory cell (SC) exhibits a watery cytoplasm where secretory granules (G) are found. 9000

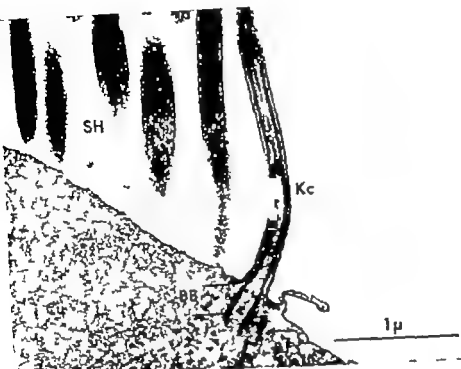


Fig. 6. Hair cell top with cuticle (C) and sensory hairs (SH). There is completely developed kinocilium (K) connected to basal body (BB) - the part of the cytoplasm free of cuticle. In the posterior part of the basal body basal foot (BF) is regularly present. 23,000.

On leaving the cartilage the nerve fibers lose their myelin sheath spread fanwise along the basilar membrane and turn upwards under the sensory cells, at the bottom of which they form nerve endings.

It has been proposed that small unmyelinated nerve fibers innervate the secretory cells of the tegmentum vasculosum (Kolmer 1928).

### *Electron Microscopy*

#### *Sensory hairs and cuticle*

The top of the hair cell is partly covered by a dense substance forming the cuticle, and located just within the plasma membrane of the flat top of the cell. About 100 sensory hairs protrude from the cuticle (Fig. 6). The posterior part of the hair-cell top has no cuticle in this region there is a basal body or centriole from which protrudes a kinocilium of varying length and development (Fig. 6). The sensory hair which is about  $0.2 \mu$  in diameter is composed of a central fine fibrillar core that is slightly denser at the periphery where it is surrounded by a plasma membrane of the cell (Fig. 7). The axial core of the hairs forms a rootlet, which continues into the cuticle. The development of the kinocilium is most advanced in the



FIG. 7 The sensory hairs are covered by the plasma membrane of the cell and end in a club-shaped swelling (a rows). The tectorial membrane (TM) adheres closely to the distal part of the hairs, which, as compared with mammals are much bent.  $\times 30,000$

long hair cells in the anterior part of the sensory epithelium. In the anterior short cells the kinocilium is sometimes rudimentary, forming only an extremely short protrusion from the basal body. Each kinocilium proper is composed of a central core with a central fibril bundle composed of nine peripheral double tubular filaments and two central tubular filaments. Each of the former continues down into the cytoplasm of the sensory cell, where another short tubular filament is added to, thus forming the centriole or basal body. A basal foot is found on the posterior side of the basal body (Fig. 6). At its base the plasma membrane covering the surface of the kinocilium is continuous with the plasma membrane of the hair cell top. The hair bundle protrudes into dome-shaped cavities on the lower surface of the tectorial membrane, with which each hair top is in close contact.

#### *The sensory cell body*

The slightly ovoid nucleus of the sensory cell is located at a constant distance from the cell surface. It is thus located in the bottom of the short sensory cells; the size of the infra-nuclear region, however, increases with the length of the cell (Figs. 8-10).

The cytoplasm has the usual organelles, with an abundance of rod-shaped

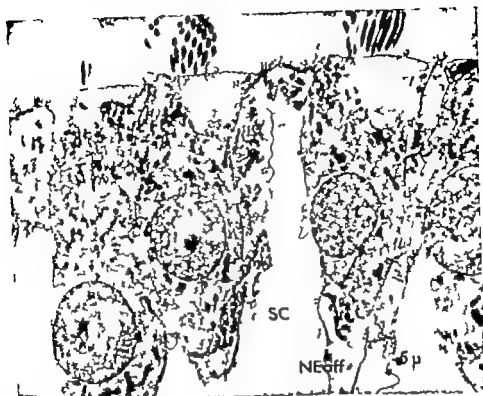


FIG. 8. The hair-cell body exhibits the cellular organelles normally present with the base of mitochondria; the basal and bottom part of the cell. Large tub-shaped afferent endings (VE II) are present with condensation at the synaptic contact and glomerular synaptic body in the hair cell (arrows). Supporting cell (SC). 5000

mitochondria in the apical cytoplasm and the synaptic region. These mitochondria have a diameter of about  $0.25 \mu$  and a length of about  $1 \mu$ . The mitochondrial cristae are transversely arranged.

Ribosomes and glycogen granules are scattered throughout the cytoplasm, and lysosome-like inclusions are found in the apical cytoplasm, together with a rich endoplasmic reticulum of the granulated type. The Golgi apparatus, with its membranes, vesicles and vacuoles, is also found in this region.

The cytoplasmic membrane is smooth, and has a "tight junction" where it makes contact with the supporting cells close to the fluid surface. This junction is followed by an intermediate one and desmosomes as described originally by Farquhar & Palade (1963) and in the inner ear by Lundquist (1963).

#### *The synaptic region*

Both afferent and efferent nerve endings are in contact with the base of the sensory cells. At the synaptic contact between the afferent nerve ending and the sensory cell a synaptic body is found. This is spherical in shape, and about  $0.2 \mu$  diameter and is in close contact with the plasma membrane.



FIG. 9. A short hair cell with basally located nucleus and numerous mitochondria (NEff) 9000



FIG. 18. The hexagonal appearance of the hair cell is apparent in cross-section. 4500.



FIG. 9. A short hair cell with basally located nucleus and prominent nucleolus (NE) (9000 $\times$ ).





Fig. 12. Afferent (VE aff) and efferent (VE eif) nerve-endings in synaptic contact with hair cell (HC). Note the dense accumulation of synaptic vesicles in the afferent ending.  $\times 12,000$ .



Fig. 11 The nerve endings are often large and club-shaped. In this efferent ending (NE aff) the synaptic contact is recognized by a condensation in the cytoplasm at both the nerve ending and at the side of the hair cell. Here a globular synaptic body (SB) is found surrounded by synaptic vesicles. Hair cell (HC) 16,500

The synaptic body is surrounded by a dense accumulation of synaptic vesicles (Fig 11). Similar bodies have been described in the synaptic region of vestibular sensory cells in fishes (Wersäll & Flock, 1962) and frogs (Gleasoner *et al* 1967). Close to the plasma membrane on both the hair-cell and nerve-ending sides of the synaptic space there is a thin layer of an electron-dense substance (Wersäll *et al* 1967).

The afferent nerve endings contains a few scattered vesicles, mitochondria and nerve fibrils.

In the synaptic region of the efferent nerve ending the hair cell is provided with a synaptic cleft. This is contributed by a synaptic sac formed by a membrane bound flat space located close to the synaptic membrane. There is also a condensation of the cytoplasm at the synaptic contact (Fig 12). A synaptic sac of this type was originally described by Engström & Wersäll (1955) in the cochlear hair cells of the guinea pig and has since been found in the synaptic area of the efferent endings in all sensory epithelia of the acoustico-lateralis system (Wersäll 1956, Wersäll & Flock 1960, Wersäll *et al* 1967).

The efferent nerve ending is filled with synaptic vesicles and is thus similar in structure to that found in mammals, fishes and amphibia.

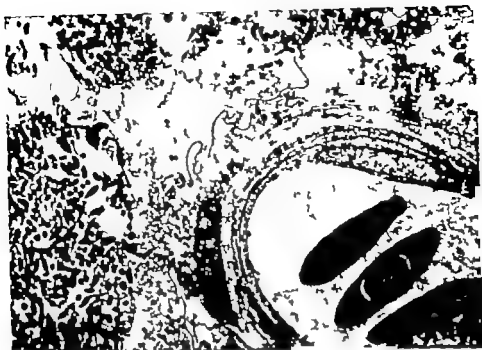


Fig. 14. The tegmentum vasculosum consists of dark cells with a number of cytoplasmic projections filled with mitochondria. The light cells, on the other hand, interdigitate with these, more villiform processes between the other a rich capillary network is seen.  $\times 4000$

### *Tectorial membrane*

The tectorial membrane is composed of densely packed fibrils with a diameter of about 100 Å, embedded in an amorphous ground substance. These fibrils are oriented with a main axis in the antero-posterior direction.

### *Tegmentum vasculosum*

The tegmentum is composed of dark and light cells. The dark cells form the apical part, reaching the surface of tegmentum, with a constricted neck and a more irregularly shaped basal part containing the nucleus. This bottom part gives off long protrusions, which digitate with ones from the light cells. The nucleus of the dark cells, which is slightly irregular in shape, contains a fairly dense chromatin network. The cytoplasm is extremely dense, filled with packed mitochondria of various shapes and lengths. The plasma membrane forms multiple folds into the cell to increase the surface area. There is a special concentration of the rod-shaped mitochondria in these infoldings. The structure of the dark cell in the tegmentum of the cock is very similar to that in the pigeon as described by Dohleman *et al* (1959).

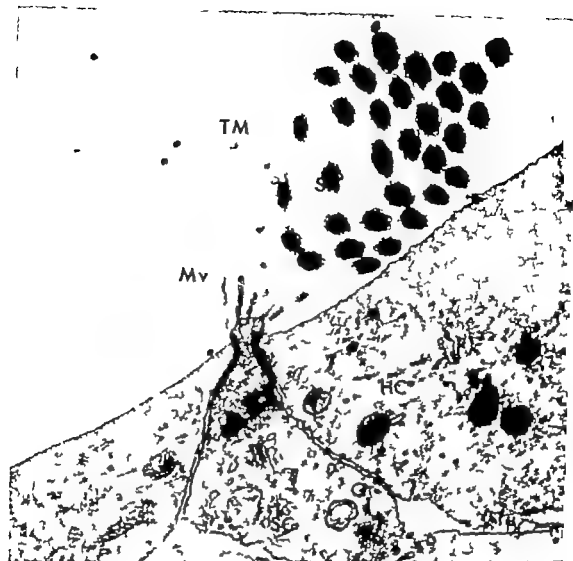


FIG. 13 The secretory supporting cell (SC) has a tuft of microvilli (Mv) at the cell surface. These villi are in close contact with strands of fibrillar material from the tectorial membrane (TM). A centriole (C) and granules are found in the cytoplasm. Hair cell (HC) with transected sensory hairs (SH).  $\times 20,000$ .

### The supporting cells

The cytoplasm of the supporting cells seems to contain a large amount of water. The cytoplasmic organelles are scattered throughout the cell with a few mitochondria, endoplasmic reticulum granules and vesicles, most of which seem to be concentrated in the apical portion of the cell. The cell surface facing the endolymph bulges slightly above the surface of the neighbouring sensory cells. A tuft of microvilli approximately  $0.5 \mu$  long and  $500 \text{ \AA}$  in diameter protrudes from the cell surface. Each microvillus has a condensed core. The microvilli are in close contact with the strands of amorphous and fibrillar material connecting the supporting cells with the lower surface of the tectorial membrane. The appearance of secretory granules in the apical portion of the supporting cells suggests that they are concerned in the formation of the matrix of the tectorial membrane.

electrodes, Nelson Kiang found inhibition of the spontaneous activity of the nerve fibers when the basilar membrane of the organ of Corti was moved towards the scala tympani, whereas movements in the opposite direction produced an increase in nerve activity. There is convincing evidence that an in-and-out motion of the oval window will result in an antero-posterior shearing motion between the tectorial membrane and the sensory epithelium of the basilar papilla in birds. This would mean that posterior displacement of the sensory hairs would result in an increase in activity whereas displacement in the opposite direction would produce an inhibitory effect. According to the morphological findings the kinocilium of the hair bundle is always located on the posterior side of the hair bundle. A displacement of the sensory hairs is the adequate stimulus for the sensory cell.

It was originally shown by Löwenstein & Wersäll (1959) and discussed by Löwenstein *et al* (1964) that a shearing force acting upon the sensory hairs of the vestibular sensory epithelia in the ray increases the nerve activity when the force is directed towards the kinocilium with an increased activity in the innervating nerve fibers and that an inhibition takes place when a force acts in the opposite direction. The present study indicates that the same might be true for the basilar papilla of birds. The actual importance of the kinocilium of the sensory cells for the stimulation of the sensory cells has been discussed elsewhere. It is clear that animals provided with mechano-receptors, where all sensory hairs are structurally similar to kinocilia, must depend on a transducing mechanism in which the mechanical energy is transformed to the sensory cell by the kinocilia. The fact that organ of Corti in the adult mammal has no kinocilia and that some sensory cells even on the basilar papilla have only rudimentary kinocilia, if any indicates that the kinocilia are not essential to these organs for the transformation of the mechanical energy to the sensory cells. Wersäll (1967) has suggested that the site of the kinocilium and the centriole merely indicates a morphological polarization at the molecular level that is closely linked to the physiological directions of the transducing mechanism of the cells.

The appearance of the synaptic apparatus, which is similar in all mechano-receptors of the acoustico-lateralis system, and is composed of a synaptic complex with synaptic membrane, bodies and vesicles, indicates that the synaptic complex is of great significance for the transformation of sensory stimulation on the nerve endings and nerve fibers. It is likely that during stimulation of the sensory cell a transmitter substance is released and that the site of this transmitter substance is closely related to the synaptic complex (Minnik *et al* 1963; Wersäll *et al* 1967).

Although it is difficult to draw further conclusions from the present studies to the relation between the peripheral and the central part of the hearing organ in birds for the analysis of the sound, the primitive organization of the basilar papilla, even at the ultrastructural level, clearly supports Schwannschöpfung's view (1937, 1938, 1960) that the central analysis must be of great importance in birds than in mammals.

## DISCUSSION

The sensitivity to and frequency discrimination of pure tones in many birds differs slightly from that of mammals (Heise, 1953 Knecht 1940 Schwartzkopff 1960). Morphological and evolutionary studies of the papilla basilaris in birds indicate, however that their peripheral sense organ of hearing is much less well developed than that of mammals. The sensory cells are far less well organized in the papilla basilaris of birds than in the organ of Corti of mammals. Although a difference in length of the cells can be observed there is no structural differentiation that would justify the recognition of two sensory cell types in the basilar papilla. The sensory cells themselves are most similar in structure to the type II sensory cells of the vestibular epithelia in birds and mammals and to the only known cell type in the vestibular sensory areas of fishes. The random distribution of the cell organelles contrasts with the organization of the mitochondria and the fenestrated membranes of the stellar rod shaped outer hair cell in the mammalian cochlea.

The kinocilium in cochlear hair cells of mammals is found only in the early developmental stage of these cells, and it normally disappears in adult animal (Kikuchi *et al.*, 1965 Kimura, 1966 Versali & Flock, 1966). The fact that a kinocilium is to be found in the sensory cells of the papilla basilaris, even in the adult animal indicates that these are to be looked upon as more primitive cells than those of the organ of Corti in mammals. These findings further support Löwenstein's view expressed in 1956, according to which the actual linkage between the sensory epithelia and the outer air will determine whether a sensory epithelium will respond to sound or gravitational stimuli. The bulky appearance of the basilar membrane and the basilar papilla suggests that the basilar papilla would respond poorly to travelling waves. It has been demonstrated by Békésy (1944) however that a mechanical frequency analysis based on the principle of travelling waves in the basilar papilla actually takes place even in the bird. This was in fact suggested by Hasse (1867) a century ago. There is a reason to believe that a vibration of the columella will give rise to a shearing force that affects the sensory cells and the tectorial membrane mainly in anterior-posterior direction. This shearing force would act on the sensory hairs of the hair cells, which in turn regulate the activity in the innervating nerve fibers. Apparently of major significance for the understanding of the function of the organ of hearing is Schwartzkopff's (1958) discovery that the direction of movement of the basilar membrane is closely related to the formation of the cochlear microphonic recorded from the neighbourhood of the sensory epithelium. Only a movement of a basilar membrane towards the oval window will stimulate nerve fiber activity and Schwartzkopff's (1958) finding that a movement in the opposite direction inhibits the nerve activity would seem to be of particular importance. This is true also of the basilar membrane movement in the cochlea of the cat where by analysis of a great number of nerve fibers of the acoustic nerve studied with micro

electrodes, Nelson Klang found inhibition of the spontaneous activity of the nerve fibers when the basilar membrane of the organ of Corti was moved towards the scala tympani, whereas movements in the opposite direction produced an increase in nerve activity. There is convincing evidence that an in-and-out motion of the oval window will result in an antero-posterior shearing motion between the tectorial membrane and the sensory epithelium of the basilar papilla in birds. This would mean that posterior displacement of the sensory hairs would result in an increase in activity whereas displacement in the opposite direction would produce an inhibitory effect. According to the morphological findings the kinocilium of the hair bundle is always located on the posterior side of the hair bundle. A displacement of the sensory hairs is the adequate stimulus for the sensory cell.

It was originally shown by Löwenstein & Versäll (1959) and discussed by Löwenstein et al. (1964) that a shearing force acting upon the sensory hairs of the vestibular sensory epithelia in the ray increases the nerve activity when the force is directed towards the kinocilium with an increased activity in the innervating nerve fibers and that an inhibition takes place when a force acts in the opposite direction. The present study indicates that the same might be true for the basilar papilla of birds. The actual importance of the kinocilium of the sensory cells for the stimulation of the sensory cells has been discussed elsewhere. It is clear that animals provided with mechano-receptors, where all sensory hairs are structurally similar to kinocilia, must depend on a transducing mechanism in which the mechanical energy is transformed to the sensory cell by the kinocilia. The fact that organ of Corti in the adult mammal has no kinocilia and that some sensory cells even on the basilar papilla have only rudimentary kinocilia, if any, indicates that the kinocilia are not essential to these organs for the transformation of the mechanical energy to the sensory cells. Versäll (1967) has suggested that the site of the kinocilium and the centriole merely indicates a morphological polarization at the molecular level that is closely linked to the physiological direction-sensitive transducing mechanism of the cells.

The appearance of the synaptic apparatus, which is similar in all mechano-receptors of the acoustico-lateralis system, and is composed of a synaptic complex with synaptic membrane, bodies and vesicles, indicates that the synaptic complex is of great significance for the transformation of sensory cell action on the nerve ending and nerve fibers. It is likely that during stimulation of the sensory cell a transmitter substance is released and that the site of this transmitter substance is closely related to the synaptic complex (Vinilkov et al. 1963; Versäll et al. 1967).

Although it is difficult to draw further conclusions from the present studies as to the relation between the peripheral and the central part of the hearing organ in birds for the analysis of the sound, the primitive organization of the basilar papilla, even at the ultrastructural level, clearly supports Schwartzkopff's view (1937, 1948, 1960) that the central analysis must be of greater importance in birds than in mammals.

## REFERENCES

- BÉKÉSY G. von, 1944 Über die mechanische Frequenzanalyse in der Schnecke verschiedener Tiere. *Akust. Zeitschr.* 9: 3.
- BURDET H. M. DE, 1934 Vergleichende Anatomie des stato-akustischen Organs. a. Die innere Ohrkapsel. In *Handb. d. vergl. Anat. d. Wirbeltiere* (ed. L. Bolk, et al.) 2. h. 2 Hft. Urban & Schwarzenberg, 1293.
- CORDIER, H. 1964 Sur la double innervation des cellules sensorielles dans l'organe de Corti du Pigeon. *C. R. Acad. Sci. (Par.)* 258: 6238.
- DOHLMAN G. F., ORMEROD, F. C., and McCLARY, H. 1959 The secretory epithelium of the internal ear. *Acta Otolaryng. (Stockh.)* 50: 243.
- FARQUHAR, M. B. and PALADE, G. E., 1963 Junctional complexes in serial epithelia. *J. Cell Biol.* 17: 375.
- FRIEKE, H. A. 1953 Das Gehörorgan der Vögel. *Z. d. Martin-Luther Univ. Math. Nat. w. Reihe* 2: 5: 267.
- GLEISNER, L., LUNDQUIST P.-G. and WERNALL, J. 1967: Synaptic structures in the vestibular end organ of frog. *Proc. Scand. Soc. Electron Microsc. J. Ultrastruct. Res.* 18: 231.
- HASSE, C., 1867: Die Schnecke d. Vögel. *Z. Wiss. Zool.* 1: 56.
- HENKE, G. A., 1953 Auditory thresholds in the pigeon. *Amer. J. Psychol.* 116: 1.
- HELD, H. 1928 Die Cochlea der Säuger und der Vögel, ihre Entwicklung und ihr Bau. In *Handb. d. normal u. pathol. Physiol.* (ed. von Bethe et al.) 11: 467. Berlin.
- LIANG, Y. Y. S. 1965 Discharge patterns of single fibers in the cat's auditory nerve. Research Monograph no. 35. MIT Press, Cambridge Mass.
- KIECHT, K. and HILDEBRAND, D. A. 1963: The development of the organ of Corti in the mouse. *Acta Otolaryng. (Stockh.)* 60: 207.
- KIMURA, R. S. 1966 Hairs of the cochlea sensory cells and their attachment to the tectorial membrane. *Acta Otolaryng. (Stockh.)* 61: 35.
- KNECHT, S. 1940 Über den Gehörsinn und die Musikalität der Vögel. *Z. vergl. Physiol.* 27: 169.
- KOLMER, W. 1928: Über die Innervation des Tegmentum vasculum d. Vogellabyrinthes. *Anat. Anz.* 66: 1: 42.
- LOWENSTEIN, O. and WERNALL, J. 1939: A functional interpretation of the electron-microscopic structure of the sensory hairs in the isthmus of the basilar branch of the cochlea in terms of directional sensitivity. *Nature* 144: 1807.
- LOWENSTEIN, O., OSBORNE, M. P. and WERNALL, J. 1964: Structure and innervation of the sensory epithelia of the labyrinth in the Thick-kay (Raja clavata). *Proc. R. Soc. (Biol.)* 160: 1.
- LUFT, J. H. 1961 Improvement in poly resin embedding methods. *J. Biophys. Biochem. Cytol.* 9: 409.
- LUNDQUIST P.-G. 1965 The endolymphatic duct and sac in the guinea pig—an electron-microscopic and perimetric investigation. *Acta Otolaryng. (Stockh.)* 5: suppl. 401.
- NETSCHE, H. 1884 Das Gehörorgan der Wirbeltiere II. Das Innenohr der Reptilien, der Vögel und der Säuger. Samson und Wallin, Stockholm.
- RHODIN, J. 1954 Correlation of ultrastructural organization and function in normal and experimentally changed peripheral conducted tube cells of the mouse kidney. Thesis. AB Godvill, Stockholm.
- SCHWARTZ, J. 1957 Untersuchung der akustischen Kerne in der Medulla Wessertischen mit Hilfe des Elektronenmikroskops. *Z. f. Vergleich. Anat. u. Physiol.* 37: 374.
- 1958 Über den Einfluss der Bewegungsrichtung der Basalmembran auf die Ausbildung der Cochlea Potentiale von Strix asiatica (Bart.) und Melospiza melodia (Shaw). *Z. vergl. Physiol.* 43: 35.
- 1960 Physiologie der höheren Hirnbelhörungs- und Hörorgane. *J. Ornithol.* 101: 1: 61.
- SCHWARTZ, J. and WERNER, P. 1960 Zu Anatomie der Vogelschnecke unter natürlichen Bedingungen. *Biol. Zbl.* 79: 607.



- STRANDBERG, E. 1931. Vog. L. In K&H's *Handbuch d. Zoologie* 7: 124.
- WIMMILL, J. 1966. Studies on the structure and innervation of the sensory epithelium of the cristae ampullares of the guinea pig. 4. *J. Otolaryng.* (Stockh.) 5: ppl. 126-127. I. Submicroscopic structure of the inner ear (ed. S. Furut.) Pergamon Press.
- WIMMILL, J. and FLOCK, A. 1962. A study of the fine structure of the sensory hairs of the receptor cells in the lateral line organ of fish, with special reference to the function of the receptors. *J. Cell Biol.* 15: 19.
- WIMMILL, J. FLOCK, A. and LUNDQVIST P.-G. 1965. Structural basis for directional sensitivity of cochlear and vestibular sensory receptors. *Cold Spring Harbor Symp. Quant. Biol.* 30: 115.
- WIMMILL, J. and FLOCK, A. 1966. Morphological aspects of cochlear hair cell physiology. I. *Henry Ford Hospital Int. Symp. on Sensorineural Hearing Processes and Disorders* (Detroit) Academic Press, New York.
- WIMMILL, J. GLENNER, L. and LUNDQVIST P.-G. 1967. Ultrastructure of the vestibular end organs. I. *Metabolic, Electrophysiological and Vestibular Mechanisms*. Ciba Foundation Symposium (ed. J. V. B. de Ruyck) p. 104.
- TRYKOR, I. A., OLSOVA, I. V., TITOVA, L. K., and GORODNEVA, V. L. 1965. Electron microscopy of the Corti organ of birds. *Zh. Obshch. Biol.* 26: 138.

Dept. of Otolaryngology  
Karolinska sjukhuset  
Stockholm, Sweden

Received May 15, 1968

## ACTIVITY OF ACETYLCHOLINESTERASE ON THE ENDOLYMPHATIC SURFACE OF OUTER HAIR CELLS

Y. KANEKO and J. F. DALY

*From the Department of Otorhinolaryngology New York University School of Medicine New York N.Y. U.S.A.*

By means of the Karnovsky's modification of Koelle's thiocholine method for acetylcholinesterase (AChE) it has been demonstrated electronmicroscopically that AChE is located on the endolymphatic surface of outer hair cells and not on the supporting cells in the organ of Corti of the guinea pig cochlea. The tissue from the middle part of the basal turn was used in all experiments. The identification of AChE was confirmed by use of appropriate concentration of selective inhibitors of cholinesterase. From the inhibitory effect of fixatives on AChE activity it was obvious that the activity on the surface of outer hair cells was lower than the activity on the large nerve endings at the bottom of the hair cells.

The surface of the sensory hair cells of the organ of Corti has been of great interest because of its potential as a functional interphase between the endolymph and the hair cells. Tasaki *et al* (1952) suggested that the upper part of the surface membrane of the hair cells plays an important role in generating the cochlear microphonic. Vinnikov & Titova (1964) demonstrated with light microscopy acetylcholinesterase activity in the hairs of the inner and outer hair cells. The authors suggested that the surface of the hair cells may be a special type of postsynaptic membrane. Recently histochemical techniques have been combined with electronmicroscopy in order to localize enzyme activity in specific structures of individual cells. The present study has employed the combined technique to investigate acetylcholinesterase activity on the endolymphatic surface of the outer hair cells of the organ of Corti.

### METHOD

The temporal bones of normal guinea pigs were obtained immediately after sacrifice and placed in a cold fixative solution. Two fixatives were used, (1) 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2) and (2) 4% formaldehyde buffered with 0.1 M of the same buffer including 4.0% sucrose. During fixation the bony capsule was removed and the tissue

This research was supported by grant NB02517 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

of each turn of the cochlea was separated. The duration of fixation was determined after studying the effects of fixation time on the enzyme activity. Fixation time varied up to 4 hours for glutaraldehyde and up to 27 hours for formaldehyde. A maximum fixation time of 23 minutes was selected for glutaraldehyde and 4 hours for formaldehyde.

The tissues were incubated in Karnovsky's media (1964) for acetylcholinesterase activity. This consisted of 50 ml of sodium citrate (1 M), 10 ml of copper sulfate (30 mM), 10 ml of water, 10 ml of potassium ferrieyanide (5 mM), 5 g of sucrose and 0.5 ml of acetylthiocholine as substrate. Incubation was carried out at 4°C for 15 minutes.

A second set of experiments was designed to show the effect of specific inhibitors on acetylcholinesterase activity. The tissue was first incubated in the phosphate buffer solution for 25 minutes to which eserine  $1 \times 10^{-4}$  M or BW 62C47  $5 \times 10^{-4}$  M had been added and then transferred to the complete media with the substrate and the same amount of inhibitors (BW 62C47 was supplied by Burroughs Wellcome Co). Controls were run by incubating the tissue in the media without the substrate acetylthiocholine. After incubation the tissue was washed briefly in 0.2 M sucrose buffered solution and post fixed for 1 hour in 1% OsO<sub>4</sub> buffered with veronal acetate. The tissue was dehydrated in a graded series of ethanol solutions and embedded in Epon. The tissue was sectioned on an LKB microtome and studied with a Siemens Elmiskop 1. Most of the sections were stained slightly with uranyl acetate and lead citrate to enhance contrast.

## RESULTS

In all of the experiments only the outer hair cells, supporting cells and Hensen's cells from the middle of the basal turn of the cochlea were examined. When the tissue was incubated in the complete medium containing acetylthiocholine as substrate, small electron opaque granular reaction product appeared on the plasma membrane surface of the outer hair cells. Neither the supporting cells, nor Hensen's cells showed these deposits on their surface membrane. There were no deposits within the cytoplasm of either the outer hair cells, or the supporting cells. (Figs. 1, 2a, b, c, 3a, 4a). The reaction product was also present on the large nerve endings at the base of the hair cells. These deposits on the nerve endings had been demonstrated before (Harko & Doly 1967, in press).

In order to demonstrate that the granular products were the result of acetylcholinesterase (AChE) activity specimens of tissue were incubated in media without the substrate. In these experiments granular products were not seen on the surface of the hair cells, however finely divided precipitates were seen (Figs. 3b, 4b).

Next specimens of tissue were incubated in complete media containing substrate which had been added the inhibitors of cholinesterase eserine  $1 \times 10^{-4}$  M and BW 62C47  $5 \times 10^{-4}$  M (Fig. 4, d). In these experiments granu-



FIG 1 Tangential section through the apical surface of outer hair cells of 1st row. Endolymphatic space of hair cell including the plasma membrane of hair show the reaction products. No reaction product on the supporting cells. Prefixation with formaldehyde 45 minutes. Incubation time 10 minutes. S, supporting cell (reticular membrane) C, cuticular plate of hair cell h hair (stereocilia) on hair cells v microvilli on supporting cells.

lar products did not appear on the surface of the hair cells. It was concluded that the granular products were the result of specific cholinesterase activity. The fine precipitates appeared when the substrate was absent from the media and also in experiments in which the cholinesterase inhibitors were added. It was concluded that the fine precipitates were not due to the AChE activity but due to some other reduction reaction.

After prolonged fixation with glutaraldehyde or formaldehyde both the reaction products and the fine precipitates either diminished or did not appear at all. The reaction was completely stopped by 2.5% glutaraldehyde after 60 minutes, and almost completely inhibited by formaldehyde after 27 hours. Although the preservation of the tissue fixed in glutaraldehyde was fairly good the tissue showed the non-enzymatic precipitation of granular material within the cells as reported by Schlaepfer & Torack (1966) (Fig 5a).

## DISCUSSION

In this study it was demonstrated that the reaction products on the surface of the outer hair cells incubated in the complete incubation medium disappeared from the tissue which was incubated in medium lacking substrate. The use of eserine  $1 \times 10^{-4}$  M and BW 62C47  $1 \times 10^{-4}$  M as inhibitors of cholinesterase produced complete inhibition of the reaction products on the surface of the outer hair cells as well as on the large nerve endings at the bottom of the hair cells. The concentration of inhibitors used here are generally acceptable in histochemical work (Pearse 1961) with electron microscope (Barnett 1962 Karnovsky & Hung, 1963 Karnovsky 1964).

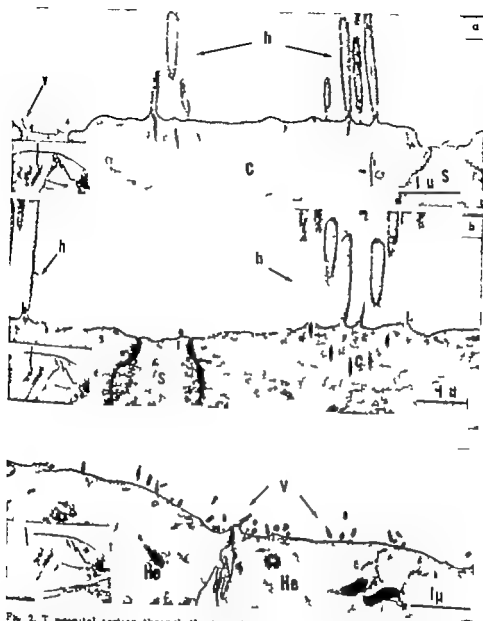


FIG. 2. T argentum section through the top of (a) outer hair cell of 1st row (b) outer hair cell of 2nd row (c) Hensen cells. Reaction products are exclusively located on the surface of the hair cell and not on the supporting cells. The surface of Hensen cells do not show the reaction products. Prefixation with formaldehyde 45 minutes. Incubation time 10 minutes.

Bloom & Barnett, 1968 Brizin *et al* 1968 Schlaepfer & Torack, 1968 Noll *et al* 1966) Following the classification of esterases given by Pearse (1966) the enzyme is either cholinesterase or an allesterase, both of which are sensitive to the concentration of eserine.

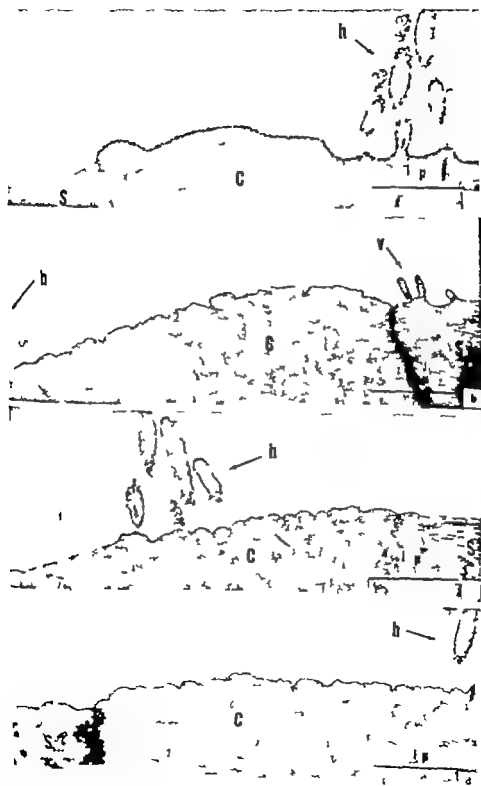


Fig. 3 (a) High magnification of reaction product on the surface of hair cell incubated in complete incubation medium show that the small granular product are continuously located on the plasma membrane of hair cells (mostly located on the plasma membrane facing inside of hair cell) (b) High magnification of precipitates on the surface of hair cells incubated in medium lacking substrate. Indefinite granular precipitates with low density are located on the surface of hair cells and not on the supporting cells. Comparing the precipitates with the reaction product of Fig. 3a it is obvious that there are differences between them in size and density. Prefixation with formaldehyde 3 hours. Incubation time 15 minutes.

Furthermore the specificity of acetylthiocholine for cholinesterase (Augustinsson 1963, Koelle 1963) and inhibition by specific cholinesterase inhibitors indicates that the reaction products on the endolymphatic surface of the outer hair cells are due to the specific cholinesterase. The reaction products did not occur in the supporting cells or Hensen's cells.

It is generally admitted that the first step in the transformation of mechanical acoustic energy into electrical energy occurs at the level of the hair cells. Although it has been established electrophysiologically that the cochlear microphonic arises in the region of the upper end of the hair cells (Tasaki *et al.* 1952) the exact place of its origin is not yet known.

In their electrophysiological experiments Tanaka & Katzuki (1966) and Katzuki *et al.* (1965) found pharmacological activity of acetylcholine and di-tubocurarine in the vicinity of the hair cells. The findings of specific cholinesterase activity on the surface of the hair cells suggests that a cholinergic mechanism is present on the endolymphatic surface of the hair cells.



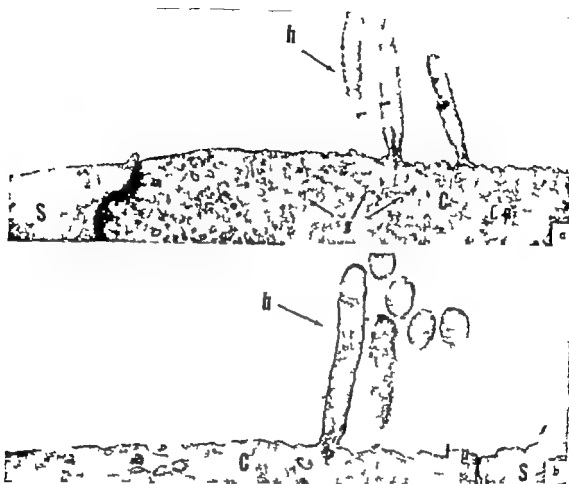


FIG. 5 (a) The tissue prefixed with glutaraldehyde for 90 minutes and incubated in complete incubation medium. Reaction products and fine precipitates on the surface of hair cells were not seen. Non-specific granular products (g) appeared in the tissue. (b) The tissue incubated in phosphate buffer—no reaction products.

When the tissue was incubated in the medium lacking the substrate there still remained fine precipitates on the endolymphatic surface of the outer hair cells, also on the hairs themselves. The formation of the fine precipitates was not affected by the cholinesterase inhibitors. It is possible that these precipitates are the result of the reduction of ferricyanide to ferrocyanide, for instance by SH group in the surface of hair cells. However this will require further investigation.

FIG. 4 (a) The tissue incubated in complete incubation medium. (b) Control preparation. The tissue incubated in medium lacking substrate (c) Inhibition experiment. The tissue treated by inhibitor eserine  $10^{-4} M$  with complete incubation medium. (d) Inhibition experiment. The tissue treated by inhibitor BW 6247 E  $10^{-4} M$  with complete incubation medium. In Figs. 4 b-d small tiny precipitates are located on the surface of hair cells including the hairs and not on the supporting cells. The localization of these precipitates is not of the reaction product of Fig. 4 a, but the density and size of these precipitates are less and smaller than for the reaction product. Prefixation with formaldehyde 2 hours. Incubation time 25 minutes.



Many investigators have reported that postfixation is necessary to maintain not only optimal structural integrity but also to obtain a sharp histochemical localization of cholinesterase (Conteaux, 1958; Lewis & Shute, 1966; Shimizu & Ishii, 1968; Shute & Lewis, 1966; Robinson & Bell, 1967; Eranko *et al.* 1967). However, when the tissue was fixed in the glutaraldehyde for more than 40 minutes the reaction products on the surface of hair cells did not appear. Koelle (1962) has reported on the inhibitory effects of glutaraldehyde on the activity of cholinesterase. On the other hand, the reaction products were still located on the large nerve endings at the bottom of the hair cells even after long fixation with glutaraldehyde (Kaneko & Daly 1967). This difference between activity on the surface of the hair cells and in the nerve endings at the bottom of the hair cells was constant. We are not able to explain this difference other than to suggest there may be a difference in the strength of the reaction of these sites. It may be that the activity of specific cholinesterase on the surface of the outer hair cells is weak and can be easily inhibited by glutaraldehyde.

#### ACKNOWLEDGMENTS

The authors wish to acknowledge the constructive criticism and helpful advice given by Dr K. Cheng. The valuable technical assistance of electronmicroscopic preparation of Mrs G. Richardson, and the histochemical preparations of Mr R. Gore are also gratefully acknowledged.

#### ZUSAMMENFASSUNG

Mit Koelle's Thiocholinmethode zum Nachweis von Acetylcholinesterase (AChE) modifiziert nach Karnovsky wurde elektronenmikroskopisch gezeigt, dass im Cortischen Organ der Meerschweinchen AChE anschiesslich auf der endolymphatischen Oberfläche der äusseren Haarzellen lokalisiert ist, nicht aber auf der Oberfläche der Stützzellen. Unsere Untersuchungen beziehen sich auf Gewebe vom mittleren Teil der Basalmembran der Cochlea. Der Nachweis von AChE wurde bestätigt durch Verwendung entsprechender Konzentrationen von Cholinesteraseinhibitor. In der Hemmungswirkung von Fixierungsmitteln auf die AChE-Aktivität der Oberfläche wurde deutlich, dass die Aktivität auf der Oberfläche der äusseren Haarzellen geringer ist als die Aktivität an den grossen Nervenendigungen im unteren Teil der Haarzellen.

#### REFERENCES

- ALLEN, W. B., 1963 *Handbook of Experimental Pharmacology* (ed. G. B. Klee) pp. 15-22. Springer Verlag, Berlin.  
 DALY, J. W., 1962 The fine structural localization of acetylcholinesterase at the neuromuscular junction. *J. Cell Biol.* 12, 217.  
 UCHIDA, F. L. and BARNETT, R. J. 1966 Fine structural localization of acetylcholinesterase: electrophoretic localization of the enzyme. *J. Cell Biol.* 29, 475.

- BREIN M, TERRAYSON V M and DUFFY P E., 1966 Acetylcholinesterase in frog sympathetic and dorsal root ganglia (a study by electronmicroscope cytochemistry and microgasometric analysis with the magnetic diver) *J Cell Biol* 31 215.
- COUTEAUX, R. 1958 Morphological and cytochemical observations on the post synaptic membrane at motor end plate and ganglionic synapses. *Exp Cell Res Suppl* 5, 294.
- ERLENK, O, REICHARDT L., and HÄKIMIAN, L., 1967 Electronmicroscopic demonstration of cholinesterase in nervous tissue *Histochemie* 8 369
- KANEKO, Y and DALY J F. Acetylcholinesterase in the nerve endings of outer hair cells and the tunnel radial fibers. Accepted for publication
- KARNOVSKY M J. 1964: The localization of cholinesterase activity in rat cardiac muscle by electron microscopy *J Cell Biol* 23 217
- KARNOVSKY M J and HUG, K., 1963 The nature of the M band enzyme in rat ventricular muscle. *J Cell Biol* 19 255.
- KATZUKI, Y, TAKAKA, Y and MINOCHI, T. 1965 Action of acetylcholine on cochlear responses. *Natur* 20 32.
- KOELLE, G B. 1963 *Handbuch der Experimentellen Pharmacologie* vol 13, p. 190. Springer Verlag, Berlin
- LEWIS, P R and SHUTE, C. C. D. 1966: The distribution of cholinesterase in cholinergic neurons demonstrated with the electron microscope. *J Cell Sci* 1 381
- NOVIKOV A B, QUINCY, A, VILLAVENDE, H and FORSCHUM, R., 1966: Anion exchange phosphatase and cholinesterase activity in dorsal root ganglia and peripheral nerve *J Cell Biol* 29 525
- PEARSE, A G E., 1961: *Histochemistry* p. 482. Little Brown & Company Boston
- ROBINSON, P M and BELL, C., 1967: The localization of acetylcholinesterase at the neuromuscular junction. *J Cell Biol* 33 93
- SCHLAEFFER, W W and TORACK, R M. 1966 The ultrastructural localization of cholinesterase in the sciatic nerve of the rat *J Histochem Cytochem* 14 369
- SHIMIZU Y and ISHII, S., 1966: Electron microscopic histochemistry of acetylcholinesterase of rat brain by Karnovsky's method. *Histochem* 6 34
- SHUTE, C. C. D. and LEWIS, P R. 1966 Electron microscopy of cholinergic terminals and acetylcholinesterase-containing neurons in the hippocampal formation of the rat *Z Zellforsch* 69 334
- TAKAKA, Y and KATZUKI, Y. 1966 Pharmacological investigations of cochlear responses and of olivocochlear inhibition *J Neurophysiol* 29 95
- TAKAKI, J D, VIL, H and LEGOUX, J. 1952 The time-pattern of the cochlear microphonics (guinea pig) as recorded by differential electrodes. *J Acoust Soc Am* 24 502.
- VAN DER VLIET A and TITOV, L. K. 1964 *The organ of Corti: Its histophysiology and histochemistry* (trans. Basil H. H. H. H.) Consultant Bureau, New York.

Dept of Otolaryngology  
New York University School of Medicine  
550 First Avenue New York  
NY 10016 U.S.A

Received August 28 1968

## THE ABSENCE OF EFFECTS OF ATARAX ON AUDITORY FATIGUE

NANCY C. WHITE and W. DIXON WARD

*From the Department of Otolaryngology, University of Minnesota School of Medicine, Minneapolis, Minn., U.S.A.*

Is a partial replication of a previous experiment by Wojnarowska, 12 normal-hearing adults between the ages of 18 and 28 were given oral 50- or 10-mg doses of atarax or either of two placebos prior to an 8-min exposure to a 1500-Hz tone at 105 dB SPL. Three control and four experimental sessions were conducted. Comparison of pre- and post-exposure thresholds for these indicated that neither the drugs nor the placebos had a significant effect on either the amount or duration of auditory fatigue.

There has been little systematic investigation of the effects of pharmacological agents on auditory fatigue, particularly as it occurs in humans (as opposed to guinea pigs). The few studies concerned with drug effects in humans vary considerably in method, drug type and dosage, and in frequency intensity and duration of fatigue-producing stimuli. Thus results are not comparable, and viewed as a body they provide no conclusive evidence as to the nature of the relation between the administration of pharmacological agents and the extent and duration of temporary shifts of hearing threshold (TTS) indicative of auditory fatigue.

For example, some attention has been focused on the effects of vitamin A. Willems (1952) exposed five normal hearing subjects to a 2000-Hz tone at 120 dB for 8 min before and after each received daily doses of 300 000 units of vitamin A. He found that four of the five demonstrated a slight reduction in the amount of TTS following the vitamin therapy. However, in a controlled study Ward & Glorig (1960) found that daily doses of 100,000 units of vitamin A given over the course of a week, had no effect on TTS. Their subjects were 15 normal hearing males who were exposed for 1 hr to octave-band noise of 2400-4800 Hz at 100 dB SPL. These authors pointed out that vitamin A is a drug whose significance for sensory processes is normally reflected by its absence and there is no reason to suppose that individuals without A vitaminosis will show improvement in sensory functioning as a result of additional doses of vitamin A.

It must be noted that have been examined by Matsui *et al* (1965). They indicate that prior to a 1-hr exposure to white noise, the administration of either estradiol or estradiol produced a significant reduction in the duration of TTS in 11 men but that the administration of testosterone had no effect

on either the amount or duration of TTS in seven women. However these authors did not clearly specify the nature of their control groups or the intensity of the fatigue-producing stimulus they used. They reported the latter only as being "90-95 phons".

Other drugs examined for a possible influence on auditory fatigue have included quinine, phenamine, and 5 phenyl 2 imin-4 oxo-oxazolidine. Womack (1961) administered three daily doses of 300 mg of quinine sulfate to seven men. He found no significant differences between this group and a control group which had received placebos, in TTS at 4000 Hz following a 7 min exposure to white noise at 117 dB SPL. Chernyak (1958) reports, though without details, that the administration of phenamine 1 hr before noise exposure allegedly produced a significant reduction in TTS in most subjects tested. Similar claims (and similarly unaccompanied by detailed data) are made for chondroitin sulphate, vitamin B<sub>1</sub> and pantothenic acid (Kubo 1963).

One of the few drugs which has been alleged to produce an increase in auditory fatigue is 5 phenyl 2 imin-4-oxo-oxazolidine. Viraglia & Amorelli (1962) have reported the results of several experiments in which a potentiation of fatigue was noted following the administration of this drug. Unfortunately only a brief summary of their work has been available in translation, and this gives no indication of the drug dosages used, the duration of its administration, or the nature of the exposure stimuli whose effects it was found to increase.

The most recent study is that of Wojnarowska (1966) who examined the effects of several stimulants and tranquilizers on auditory fatigue in 20 adults. Stimulants tested were strychnine, caffeine, and lobeline; tranquilizers tested were luminal, atarax, and meprobamate. Of these, two tranquilizers, atarax and meprobamate, and one stimulant, caffeine, were claimed to have produced a significant reduction in both the amount and duration of fatigue. She reported that following an 8-min exposure to a 1500-Hz tone, mean TTS (at an unspecified frequency) was normally 20 dB requiring 8.2 min to recover, while following administration of atarax, mean TTS was 11 dB requiring 3.6 min to recover.

Because of the striking nature of these results (both stimulants and tranquilizers showing the same effect) and because of certain methodological problems felt to be inherent in Wojnarowska's study, it was decided to conduct a partial replication of her experiment, using atarax, the tranquilizer she found to yield the most significant results. The following is a report of experimentation designed to test the effects of this drug on auditory fatigue patterns in 12 normal hearing adults.

#### METHOD

Although the experiment was intended to be a replication of Wojnarowska's work involving atarax, several modifications of her method were

affected in order to achieve a greater degree of experimental control. These concerned the intensity of the exposure stimulus, the procedure for threshold-testing before and after exposure, the drug dosage, and the manner of its administration.

Wojnarowska's exposure stimulus consisted of a 1500-Hz tone, presented for 8-min at 75 dB above a predetermined threshold (Wojnarowska, 1966). In preliminary tests for the current investigation, it was found that, for a 1500-Hz tone, the minimum intensity required to produce a significant (15 dB or more) and consistent level of fatigue in normal-hearing subjects was 105 dB SPL (98.5 dB HL ISO 1964). Consequently this was the intensity selected for use.

Wojnarowska's threshold testing was conducted with a 5-dB discrete step audiometer. In order to obtain more finely-grained measurements and to reduce the probability of experimenter error in testing, it was decided to employ Békésy audiometry. This allows for continuous and automatic recording of a subject's threshold and its variation in estimation to 1-dB accuracy. In addition, it was decided to measure post-exposure thresholds continuously for 8 min, a period equal to that of the exposure duration. It was felt that this would yield a more complete pattern of the progress of recovery than would measuring thresholds at 2 min intervals as Wojnarowska had done.

The dosage of atarax received by her subjects was 10 mg, administered orally in a single-blind procedure (Wojnarowska, 1966). According to the medical director of J. B. Roerig & Sons, the U. S. manufacturer of this drug, atarax is usually dispensed in 50-mg tablets, and the normal, daily adult dosage is four of these or 200 mg (Fowler 1968). Because of this fact, it was decided to examine the effects of both 50-mg and 10-mg doses of atarax in the current investigation, even though Wojnarowska used only 10-mg doses.

Finally to avoid experimenter bias, it was felt necessary to use a double-blind procedure in the test administration.

The subjects were five men and seven women. Their ages ranged from 18 to 26 years with a mean of 23 years. Ten were college students, and two were Hearing Research Laboratory personnel. The results of Békésy audiometry conducted within a week prior to the experiment, indicated that all had normal hearing, as defined by thresholds no greater than 15 dB HL (re ISO 1964 standard) for frequencies of 1000, 1500, 2000 and 4000 Hz. In the course of this testing, all demonstrated the ability to respond to a Békésy audiometry procedure with variation of 7 dB or less in threshold range. The subject reported themselves to be without colds, ear infections, or other ear problems. None was taking any medication at the time of the experiment.

Pure tones for testing were generated by a Hewlett Packard oscillator Model 201CR, and shaped with a Grason-Stadie 829C electronic switch to yield 250-msec tone pulses with 25-msec rise- and decay-times and a 250-

on either the amount or duration of TTS in seven women. However these authors did not clearly specify the nature of their control groups or the intensity of the fatigue producing stimulus they used. They reported the latter only as being "90-95 phons".

Other drugs examined for a possible influence on auditory fatigue have included quinine, phenamine, and 5-phenyl 2 imin-4 oxo-oxazolidine. Womack (1961) administered three daily doses of 300 mg of quinine sulfate to seven men. He found no significant differences between this group and a control group which had received placebo, in TTS at 4000 Hz following a 7 min exposure to white noise at 117 dB SPL. Chernyak (1958) reports, though without details, that the administration of phenamine 1 hr before noise exposure allegedly produced a significant reduction in TTS in most subjects tested. Similar claims (and similarly unaccompanied by detailed data) are made for chondroitin sulphate, vitamin B<sub>1</sub>, and pantothenic acid (Kubo, 1963).

One of the few drugs which has been alleged to produce an increase in auditory fatigue is 5-phenyl 2 imin-4-oxo-oxazolidine. Miraglia & Amorelli (1962) have reported the results of several experiments in which a potentiation of fatigue was noted following the administration of this drug. Unfortunately only a brief summary of their work has been available in translation, and this gives no indication of the drug dosages used, the duration of its administration, or the nature of the exposure stimuli whose effects it was found to increase.

The most recent study is that of Wojnarowska (1966) who examined the effects of several stimulants and tranquilizers on auditory fatigue in 20 adults. Stimulants tested were strychnine, caffeine, and lobeline; tranquilizers tested were luminal, atarax, and meprobamate. Of these, two tranquilizers, atarax and meprobamate, and one stimulant, caffeine, were claimed to have produced a significant reduction in both the amount and duration of fatigue. She reported that, following an 8-min exposure to a 1500-Hz tone, mean TTS (at an unspecified frequency) was normally 20 dB requiring 8.2 min to recover, while following administration of atarax, mean TTS was 11 dB requiring 3.6 min to recover.

Because of the striking nature of these results (both stimulants and tranquilizers showing the same effect) and because of certain methodological problems felt to be inherent in Wojnarowska's study, it was decided to conduct a partial replication of her experiment, using atarax, the tranquilizer she found to yield the most significant results. The following is a report of experimentation designed to test the effects of this drug on auditory fatigue patterns in 12 normal hearing adults.

#### METHOD

Although the experiment was intended to be a replication of Wojnarowska's work involving atarax, several modifications of her method were

presented in the following order: 2000, 1500, and 1000 Hz to the left ear; 1000, 1500, and 2000 Hz to the right ear; and 4000 Hz to the left ear. With the exception of the first presentation to the left ear of 2000 Hz, which was of 40 sec duration, each frequency was tested for 20 sec. The above order was repeated until a total of 8 min had elapsed. Thus, following exposure, four measurements were obtained for 2000 Hz in the left ear and three for each of the other frequencies in either ear.

Thirty minutes before each of the four experimental sessions, the subjects were given oral doses of either the drugs or the placebos. Because the 50-mg alarax was in capsule and the 10-mg in liquid (10 mg/dram) form, two placebos, one in each form, were used. The 12 subjects were divided into four groups, and each group of three received the drugs and placebos in a different order in alternating liquid and capsule forms. All doses were administered by the experimenter under the supervision of Dr Arndt J. Duvall, III. The subjects received them, followed by 4 oz. of water at least an hour after having eaten. As mentioned previously, a double-blind procedure was employed, so neither the experimenter nor the subjects knew whether they were being given drug or placebo. Prior to each administration, the subject was told the following:

This is one of the 4 sessions in which you are being given either a mild tranquilizer or a placebo. Both are in liquid and capsule forms. For today you will swallow this capsule (liquid). After 30 minutes, your hearing will be tested before and after you listen to the loud sound. During the 30-minute waiting period, you are asked not to smoke and not to eat or drink anything. If you feel drowsy at any time following the experimental session, you are cautioned not to drive and not to operate any dangerous machinery.

## RESULTS

Pre- and post-exposure threshold values were determined by visual estimation of the midpoint of the Bekesy tracings for each frequency. To obtain an indication of the reliability of these measurements, pre-session tracings for each subject were measured by the experimenter (NW) on two separate occasions, usually a day apart. Comparison of midpoint values assigned as threshold indicated maximum variation of only 1 dB in either direction for any frequency. The mean difference (disregarding sign) was 0.12 dB.

The mean of pre-exposure thresholds for all frequencies in each ear were determined, and their comparison for all sessions showed maximum variability to be 2 dB between any two sessions with a net shift of less than 1 dB. Thus neither the drug nor the placebos had any significant effect on threshold of audibility.

Mean shifts, computed for all frequencies in each ear, revealed between-session difference of less than 2 dB. Within this range, immediate post-

msc silent period. A Grason Stadler E3262A recording attenuator changed the level of the test signal at the rate of 4 dB per sec. The continuous exposure tone was generated by a second Hewlett Packard 201CR oscillator. Voltages across the earphones were calibrated for all tones with a Ballantine 300G vacuum tube voltmeter at the beginning of each day. The tones were presented through PDR8 earphones in MX-41/AR cushions in an Industrial Acoustics sound treated room model 1202A.

### PROCEDURE

Seven sessions, three control and four experimental, were conducted within a 2 wk period for each subject. Two control sessions preceded and one followed those involving the drugs and placebo. Prior to the first session, the subjects were told the following:

You are being asked to participate in an experiment designed to test the effects of 2 different, but harmless drugs on hearing. Altogether there will be 7 experimental sessions. During each, your hearing will be tested then you will be asked to listen to a loud sound for a few minutes, and then your hearing will be tested again. Before 4 of the sessions, you will be given either a mild tranquilizer or a placebo both will be in liquid and capsule forms. On 2 occasions you will receive the drug, and on 2 occasions, the placebo. These 4 sessions will take about an hour each, the other 3 sessions will take about a half hour each. Are there any questions?

For each session, the procedure for threshold testing and exposure was the same. The subject was seated in the sound treated room and was given standardized instructions which stressed (1) pressing the voting button just as soon as a single beep was heard and releasing it as soon as one was missed, (2) adjusting headphones for snug fit, and (3) being as quiet as possible while listening, e.g. by breathing through the mouth and by holding the cord to the earphones to prevent its scraping against the clothing. After making certain that the earphones were seated properly and that the subject was holding their cord as directed, testing was begun.

Thresholds were obtained first for the left ear (the ear to be exposed subsequently) and then the right. Frequencies tested were 1000, 1500, and 4000 Hz in that order. Each was presented for 30 sec. Following the tests for the right ear, the threshold in the left ear for 2000 Hz was again determined. This was to provide for immediate pre- and post-exposure comparison since this was the first threshold to be measured following exposure, and this threshold was the one which could be expected to show the greatest degree of shift. Then the exposure was begun: a 1500 Hz tone at 105 dB SPL was presented, through the earphone to the left ear for 8 min. Fifteen seconds before the end of this period, the subject was told to 'get ready' to respond to threshold testing.

Immediately after exposure testing was resumed. Frequencies were pre-



and 4000 Hz for the drug, placebo, and control sessions. For the control session data, the function is the combined mean of the second (before-exposure) and the third (after-exposure) sessions without medication. These patterns are plotted logarithmically in time in order to yield linear functions. Their examination reveals the insignificance of the differences in both TTS amount and duration following the administration of the drugs and placebos.

### DISCUSSION

These results indicate that atarax, in either 50-mg or 10-mg doses, has no effect in normal hearing listeners on the amount or duration of auditory fatigue produced by an 8-min exposure to a 1500-Hz tone at 105 dB SPL. This is in direct opposition to the striking effects in fatigue-reduction reported by Wojnarowska (1966) with 10-mg doses of atarax, followed by exposure to a similar stimulus at a presumably lower level of intensity. It is possible that atarax if administered in larger or more prolonged doses and perhaps prior to a more intense or prolonged exposure than was used in the present experiment could effect a reduction in auditory fatigue as significant as that reported by Wojnarowska, but it hardly seems likely.

Nevertheless, it may be important that further research efforts be directed toward the examination of the influence of drugs such as atarax on patterns of TTS recovery. This is especially true for drugs which could possibly reduce fatigue effects. Since repeated episodes of fatigue commonly occurring in industrial workers exposed to high levels of noise in their jobs, can lead to permanent hearing loss, pharmaceuticals which could be found to produce a reduction in fatigue might have practical significance for retarding the development of such loss.

### ZUSAMMENFASSUNG

Im Gegensatz zu Ergebnissen von Wojnarowska im Jahre 1966 vorgelegt, wurde keine Veränderung in der Hörmüdigkeit, nach einer 8-Min Aussetzung zu einem 105-dB-SPL, 1500-Hz Ton gemessen, durch entweder 10- oder 50-mg Portionen Atarax in einem doppelt blinden Verfahren produziert.

### ACKNOWLEDGMENT

The cooperation and assistance of J. R. Roerig & Sons, Division of Charles Pfizer & Son Pharmaceuticals and of its medical director J. Ralph Fowler M.D. are gratefully acknowledged. This research was supported by grant N04402 from the U.S. Public Health Service Dept. of Health, Education and Welfare.

### REFERENCES

- Cox, A. D. I. 1948 Alteration in the adaptation of the hearing to silence with relation to the duration of preliminary loud noise. *Biol. J.*, 2, 1, 13. (Transl. J. H. Ill on. 1955 *Biophysics*, 2, 1 69)

TABLE 1 *Mean initial TTS in dB in the exposed (left) ear at 2000 and 4000 Hz.*

Session	2000 Hz	4000 Hz
Control <sup>a</sup>	13.6	6.1
50-mg atarax	13.8	7.1
10-mg atarax	13.2	6.5
50-mg placebo	14.5	7.2
10-mg placebo	14.9	7.3

Mean of the second control session before the four drug placebo sessions and the control session after the four

exposure thresholds, particularly at 2000 and 4000 Hz in the exposed ear (see Table 1 for mean shifts) did tend to be slightly reduced for both of the drug sessions however differences of this magnitude do not begin to approach statistical significance. Thus, the amount of TTS was significantly affected by neither the drugs nor the placebos.

Duration of TTS was also not found to be significantly affected. Between session differences in mean final post-exposure thresholds amounted again to less than 2 dB. Here, the tendency toward higher values was noted for the drug sessions than for the control sessions, particularly for the exposed ear at 2000 and 4000 Hz. Fig. 1 illustrates TTS recovery patterns at 2000

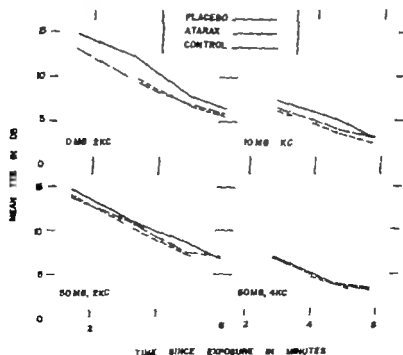


FIG. 1 Course of recovery for 8 min after exposure to drug, placebo, and control sessions. Drug dosage and test frequency are indicated for each panel. Left axis for 12 subjects.

and 4000 Hz for the drug, placebo, and control sessions. For the control session data, the function is the combined mean of the second (before-exposure) and the third (after-exposure) sessions without medication. These patterns are plotted logarithmically in time in order to yield linear functions. Their examination reveals the insignificance of the differences in both TTS amount and duration following the administration of the drugs and placebo.

### DISCUSSION

These results indicate that atarax, in either 50-mg or 10-mg doses, has no effect in normal-hearing listeners on the amount or duration of auditory fatigue produced by an 8-min exposure to a 1500-Hz tone at 105 dB SPL. This is in direct opposition to the striking effects in fatigue-reduction reported by Wojnarowska (1968) with 10-mg doses of atarax, followed by exposure to a similar stimulus at a presumably lower level of intensity. It is possible that atarax, if administered in larger or more prolonged doses and perhaps prior to a more intense or prolonged exposure than was used in the present experiment could effect a reduction in auditory fatigue as significant as that reported by Wojnarowska, but it hardly seems likely.

Nevertheless, it may be important that further research efforts be directed toward the examination of the influence of drugs such as atarax on patterns of TTS recovery. This is especially true for drugs which could possibly reduce fatigue effects. Since repeated episodes of fatigue commonly occur ring in industrial workers exposed to high levels of noise in their jobs, can lead to permanent hearing loss, pharmaceuticals which could be found to produce a reduction in fatigue might have practical significance for retarding the development of such loss.

### ZUSAMMENFASSUNG

Im Gegensatz zu Ergebnissen von Wojnarowska im Jahre 1968, wurde keine Veränderung in der Hörmüdigkeit, nach einer 8-Min Aussetzung zu einem 105-dB-SIL 1500-Hz Ton gemessen durch entweder 10- oder 50-mg Portionen Atarax in einem doppelbeliebigen Verfahren produziert.

### ACKNOWLEDGMENT

The cooperation and assistance of J. B. Roerig & Sons, Division of Charles Pfizer & Sons, Pharmaceuticals and of its medical director J. Ralph Fowler, M.D. are gratefully acknowledged. This research was supported by grant ND04403 from the U.S. Public Health Service, Dept. of Health, Education and Welfare.

### REFERENCES

- Cox, E. R. L., 1958. Alteration in the adaptation of the hearing to silence with relation to the duration of preliminary loud noise. *Biophys. J.* 3: 1-73. (Transl. J. H. Dixon, 1958. *Biophysics*, 3: 1-69.)

TABLE 1 *Mean initial TTS in dB in the exposed (left) ear at 2000 and 4000 Hz*

Session	2000 Hz	4000 Hz
Control <sup>a</sup>	13.6	6.1
50-mg atarax	13.6	7.1
10-mg atarax	13.2	6.5
50-mg placebo	14.5	7.2
10-mg placebo	14.9	7.3

Mean of the second control session before the four drug placebo sessions and the control session after the four

exposure thresholds, particularly at 2000 and 4000 Hz in the exposed ear (see Table 1 for mean shifts) did tend to be slightly reduced for both of the drug sessions however differences of this magnitude do not begin to approach statistical significance. Thus, the amount of TTS was significantly affected by neither the drugs nor the placebo.

Duration of TTS was also not found to be significantly affected. Between session differences in mean final post-exposure thresholds amounted again to less than 2 dB. Here, the tendency toward higher values was noted for the drug sessions than for the control sessions, particularly for the exposed ear at 2000 and 4000 Hz. Fig. 1 illustrates TTS recovery patterns at 2000

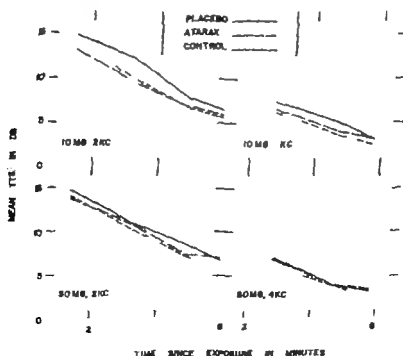


FIG. 1 Course of recovery for 8 min after exposure, in drug, placebo, and control sessions. Drug dosage and test frequency are indicated for each panel. Left ears of 11 subjects.

## RHYTHMICAL CEREBELLAR RESPONSES TO LABYRINTHINE STIMULATION

G. B. AZZERA

*From the Institute of Human Physiology, Sassari University School of Medicine  
Sassari, Italy*

The electrical activity of the cerebellar cortex has been recorded with microelectrodes simultaneously with the action potentials of a few fibers of an oculomotor nerve during eye nystagmus brought about by thermic stimulation of the labyrinth in curarized guinea pigs. Tonic and rhythmical responses to the labyrinthine stimulation were recorded from uvula, flocculus and paraflocculus. Rhythmical cerebellar responses in 81% of the cases exhibited the same frequency as that of the eye nystagmus. However in the great majority of cases the rhythmical cerebellar outbursts were not perfectly synchronous with the quick or slow phases of the eye nystagmus. In the remaining 19% of cases the frequency of the rhythmical cerebellar responses was different from that of the eye nystagmus. The conclusion may be reached that the cerebellum although not essential for the appearance of the eye nystagmus is the seat of rhythmical activity during eye nystagmus. Thus the cerebellum utilizes messages from the vestibular apparatus in order to influence and regulate the correct performance of the extra-ocular movements just as it acts on the limb movements.

The relationship of the cerebellum to ocular movements were analyzed from different points of view. Several investigations were devoted to the study of cerebellar influence on eye movements elicited by labyrinthine stimulation and especially on labyrinthine eye nystagmus (Dow & Mann, 1904; Don & Moruzzi, 1938; Mani, 1968; Magnus, 1914) and De Kleijn & Magnus (1920) stated that the cerebellum is not necessary for the appearance of all vestibular reflexes and particularly of the eye nystagmus. However further investigations showed that cerebellar asymmetrical lesions and cerebellar stimulations can modify the vestibular reflexes (Aschan *et al*, 1963; Hain & Leidler, 1912; Chambers, 1917; Chambers & Sprague, 1953; Chien *et al*, 1960; Di Giorgio, 1950; Dow, 1938; Fernández & Friedrickson, 1963; Ferraro & Barrera, 1956; Grant *et al*, 1963; Hahn, 1940; Hare *et al*, 1970, 1937; Hashino, 1921; Hoella, 1903; Lorente de No, 1931; Le Gros Clark, 1938; Low, 1918; Magoon *et al*, 1933; Maon, 1940; Miller & Laughlin, 1928; Moruzzi, 1948; Rasmussen, 1932; Schoolman & Delgado, 1958; Steinell & Di Giorgio, 1951; Spiegel & Scaf, 1941, 1942; Whitelade & Under, 1953). The cerebellum receives direct and indirect vestibular

- FOWLER, J. R., 1968: Personal communication
- KUBO, M. 1963: Acoustic trauma—its development and prevention in transport workers. Proc 16th Japan Med. Congress, Osaka
- MATSUMI, K., SAKAMOTO, H. and KOJIMA, T. 1963: Studies on the noise-induced temporary threshold shift (NITTS) at CS dip. *J. Ind. Med.* (Tokyo) 7: 415
- MIRAGLIA DEL GUIDICE, E., and AMORELLI, A. 1962: L'inibizione centrale della fatica uditiva dopo somministrazione di  $\alpha$ -fenil 2-imino-4-oxo-oxazolidina. *Arch. Ital. Laring.* 70: 145
- WARD, W., DIXON, and GLORIA, A., 1960: The relation between vitamin A and temporary threshold shift. *Acta Otolaryng.* (Stockh.) 52: 71
- WILLEMS, C., 1952: Protection contre la surdité professionnelle rôle de la vitamine A. *Acta Otorhinolaryng. Belg.* 6: 319
- WOJNAROWSKA, W. 1966: Influence exerted on auditory fatigue by some calmatives and stimulants of the central nervous system. *Acta Otolaryng.* (Stockh.) 62: 481
- WOMACK, G. J. 1961: Effect of small doses of quinine on the temporary threshold shift of hearing induced by high-energy noise exposure. School of Aerospace Medicine USAF Report 61-70

W. Dixon Ward Ph.D.  
Box 161 Mayo University of  
Minnesota Minneapolis  
Minn 55455 U.S.A.

Received August 5 1968

## RHYTHMICAL CEREBELLAR RESPONSES TO LABYRINTHINE STIMULATION

G B AZZENA

*From the Institute of Human Physiology Sassari University School of Medicine,  
Sassari Italy*

The electrical activity of the cerebellar cortex has been recorded with microelectrodes simultaneously with the action potentials of a few fibers of an oculomotor nerve during eye nystagmus brought about by thermic stimulation of the labyrinth in curarized guinea pigs. Tonic and rhythmical responses to the labyrinthine stimulation were recorded from uvula, flocculus and paraflocculus. Rhythmical cerebellar responses in 81% of the cases exhibited the same frequency as that of the eye nystagmus. However in the great majority of cases the rhythmical cerebellar outbursts were not perfectly synchronous with the quick or slow phases of the eye nystagmus. In the remaining 19% of cases the frequency of the rhythmical cerebellar responses was different from that of the eye nystagmus. The conclusion may be reached that the cerebellum although not essential for the appearance of the eye nystagmus is the seat of rhythmical activity during eye nystagmus. Thus the cerebellum utilizes messages from the vestibular apparatus in order to influence and regulate the correct performance of the extra-ocular movements just as it acts on the limb movements.

The relationships of the cerebellum to ocular movements were analyzed from different points of view. Several investigations were devoted to the study of cerebellar influence on eye movements elicited by labyrinthine stimulation and especially on labyrinthine eye nystagmus (Dow & Manni, 1964; D. W. & M. Ruzzi, 1968; Manni, 1966; Magnus (1914) and De Kleijn & Nagou (1920) stated that the cerebellum is not necessary for the appearance of all vestibular reflexes and particularly of the eye nystagmus. However further investigations showed that cerebellar asymmetrical lesions and cerebellar stimulation can modify the vestibular reflexes (Aschan *et al.* 1963; Bauer & Leidler 1912; Chambers, 1947; Chambers & Sprague, 1950; Cohen *et al.* 1963; Di Giorgio, 1950; Dow 1938; Fernández & Fredrickson, 1963; Ferraro & Barrera, 1936; Grant *et al.* 1963; Hahn, 1930; Hare *et al.* 1936, 1937; Hoshino, 1921; Koella, 1950; Lorente de No, 1931; Le Gros Clark, 1936; Lowy 1916; M. Ruzzi *et al.* 1963; Manni 1930; Müller & Laughlin, 1923; M. Ruzzi, 1948; Ruusussen, 1933; Schoolman & Delgado, 1958; Simon III & Di Giorgio, 1931; Spiegel & Sealz, 1941, 1942; Whiteside & Selden 1953). The cerebellum receives direct and indirect vestibular

- FOWLER, J R., 1968 Personal communication.
- KUDO, M. 1963: Acoustic trauma—its development and prevention in transport workers. Proc. 16th Japan Med. Congress, Osaka.
- MATSUI, R., SAKAMOTO, H. and KOJIMA, T. 1965 Studies on the noise-induced temporary threshold shift (NITTS) at C3 dip. *J Ind Med* (Tokyo) 4:15
- MIRAGLIA DEL GUIDICE, E., and AMORELLI, A., 1962 L'inibizione centrale della fatica uditiva dopo somministrazione di 3-fenil 2 imino-4-oxo-oxazolidina. *Arch Ital Laring* 70: 145
- WARD, W. DIXON and GLOBA, A. 1960 The relation between vitamin A and temporary threshold shift. *Acta Otolaryng* (Stockh.) 52, 71
- WILLEMAE, C., 1932 Protection contre la surdité professionnelle rôle de la vitamine A, *Acta Otorhina laryng Belg* 6: 319
- WOJNAROWSKA, W. 1966: Influence exerted on auditory fatigue by some calmatives and stimulant of the central nervous system. *Acta Otolaryng* (Stockh.) 81: 481
- WOMACK, G. J., 1961: Effect of small doses of quinine on the temporary threshold shift of hearing induced by high-energy noise exposure. *School of Aerospace Medicine USAF Report 61-70*

W. Dixon Ward Ph.D  
Box 461 Mayo, University of  
Minnesota: Minneapolis,  
Minn. 55455 U.S.A.

Received August 3 1968



nystagmus, which could have been recorded if the animal had not been curarized.

The recorded sites in the cerebellum were marked by electrolysis. At the end of the experiments the animals were killed with a large dose of Nembutal. The cerebellum was removed, fixed in alcohol, embedded in paraffin, serially cut and stained with the Nissl's method.

## RESULTS

### *Eye nystagmus elicited by thermic stimulation of the labyrinth*

Before curarizing the animals, at the beginning of the experiments, the ocular effects of the thermic stimulation of the labyrinth were controlled. The cold (10 C) stimulation of a labyrinth provoked an ipsiversive slow deviation followed by eye nystagmus with the quick phase towards the contralateral side. The warm (40-45 C) irrigation of the labyrinth induced an eye nystagmus with the quick phase directed towards the stimulated side. All such results are altogether in agreement with those obtained by other investigators (Camia, 1928).

### *Electrical activity of the cerebellum during thermic stimulation of the labyrinth*

The results of the present report are based on the analysis of the records obtained in 23 guinea pigs with 24 histologically checked localizations of the recording microelectrode tip in the cerebellar cortex. The tip of the microelectrode was located in the region of the Purkinje cells. Fourteen out of the 24 localizations were in the uvula (Larsell's lobule IX), seven in the paraflocculus (H. VIII b, ILIX) and three in the flocculus (H.X.) (Fig. 1). The thermic stimulation of the labyrinth induced in all the records mod-

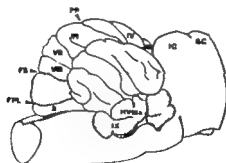


FIG. 1. Lateral view of the cerebellum of the guinea pig. FP, fissura prima; FX, fissura secunda; FPL, fissura postero-lateralis; IC, SC, inferior and superior cerebelli. The roman figures indicate the cerebellar folia according to the classification by Larsell. The dotted lines show the cerebellar lamellae (IX, I, etc.); H.X., Flocculus; H. VIII b, Paraflocculus dorsalis, and IL IX, Paraflocculus ventralis from which the rhythmical responses to the labyrinthine stimulation were recorded.

afferents, which end particularly in the flocculo-nodular lobe, lingula, uvula, paraflocculus and fastigial nuclei (Brodal *et al* 1962 Dow & Moruzzi, 1958 Jansen & Brodal, 1954) On the other hand cerebellar fibers impinge upon brain stem structures—vestibular nuclei, reticular formation, oculomotor nuclei—which are involved in the ocular labyrinthine reflexes (Azzena & Giretti, 1967 Azzena *et al* 1967 Brodal *et al.*, 1962 Carpenter & Strominger 1964 Chatelier & Leitner 1962 Duensing & Schaefer 1957 Dumont Tye & Dell 1961 Gernandt 1964) Electrophysiological studies have confirmed and extended such anatomical findings. Various stimulations of the labyrinth or of the vestibular nerve induced modifications of the cerebellar electrical activity which were particularly evident in the vestibular areas of the cerebellum (Andersson & Gernandt, 1954 Arduini & Pompelano, 1957 Camis, 1919 Chang & Kostyuk, 1960 Dow 1939 Gualtierotti & Passerini, 1958 1959 Price & Spiegel 1937 Riva Sanseverino & Urbano 1965 Weber & Steiner 1965)

The present paper was devoted to analyzing the changes in electrical activity of the cerebellum during vestibular eye nystagmus. It shows that under particular conditions the cerebellum exhibits a rhythmical activity in relationship to vestibular eye nystagmus

### METHOD

The present experiments were carried out on 52 guinea pigs weighing 400–500 g Under ether anesthesia the animals were tracheotomized, cannulated and put in a stereotaxic apparatus. Following an appropriate craniotomy the cerebellum was exposed and protected by warm mineral oil The frontal poles were also exposed and ablated by suction in order to visualize the oculomotor nerve before it entered the orbit Both epitympanic recesses were opened and cannulated in order to stimulate the labyrinth by means of warm or cold water irrigations The ether anesthesia was stopped and all the operatory wounds were infiltrated by procaine Then the animals were immobilized by d tubocurarine (1 mg i p) and the artificial respiration was begun The cerebellar electrical activity was picked up by means of tungsten microelectrodes The tip diameter of the microelectrodes was about 3–6  $\mu$  they were introduced into the cerebellum by means of a microcontrol The microelectrodes were connected through a Grass high impedance probe 5A and a P5CR preamplifier with the lower beam of a Tektronix 502 A oscilloscope

Simultaneously the electrical activity of an oculomotor nerve was recorded by means of another microelectrode connected through a Grass P5CR preamplifier with the upper beam of the oscilloscope In this way the simultaneous record of the cerebellar electrical activity and of the action potentials of a few fibers of an oculomotor nerve gave the possibility to correlate the cerebellar effects of the labyrinthine stimulation to the eye

nystagmus, which could have been recorded if the animal had not been curarized.

The recorded sites in the cerebellum were marked by electrolysis. At the end of the experiments the animals were killed with a large dose of Nembutal. The cerebellum was removed, fixed in alcohol, embedded in paraffin, serially cut and stained with the Nissl's method.

## RESULTS

### *Eye nystagmus elicited by thermic stimulation of the labyrinth*

Before curarizing the animals, at the beginning of the experiments, the ocular effects of the thermic stimulation of the labyrinth were controlled. The cold (10 C) stimulation of a labyrinth provoked an ipsiversive slow deviation followed by eye nystagmus with the quick phase towards the contralateral side. The warm (40-45 C) irrigation of the labyrinth induced an eye nystagmus with the quick phase directed towards the stimulated side. All such results are altogether in agreement with those obtained by other investigators (Camls, 1928).

### *Electrical activity of the cerebellum during thermic stimulation of the labyrinth*

The results of the present report are based on the analysis of the records obtained in 23 guinea pigs with 24 histologically checked localizations of the recording microelectrode tip in the cerebellar cortex. The tip of the microelectrode was located in the region of the Purkinje cells. Fourteen out of the 24 localizations were in the uvula (Larsell's lobule IX); seven in the paraflocculus (H. VIII b H.IX) and three in the flocculus (H.X) (Fig. 1). The thermic stimulation of the labyrinth induced in all the records mod-



FIG. 1. Lateral view of the cerebellum of the guinea pig. FP fissura prima; FS fissura secunda; FPL, fissura postero-lateralis; IC, SC, inferior and superior cerebellar peduncles. The figures indicate the cerebellar folia according to the classification by Larsell. The dotted area shows the cerebellar lamellae. IX, I is H.X, Flocculus; II VIII b Paraflocculus dorsalis, and II, IX, Paraflocculus ventralis (from which the rhythmic responses to the labyrinthine stimulation were recorded).

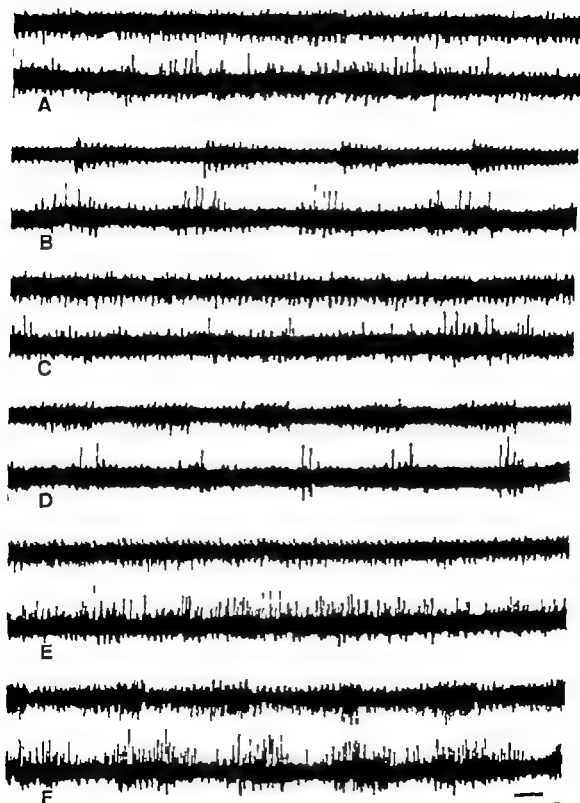


FIG. 2. Simultaneous record of the electric activity of the left oculomotor nerve (upper beam) and of the unitary discharge of the right half of the n.ula (lower beam) Guinea pig no 490 (A) Normal electrical activity resting condition. (B) Immediately after a warm stimulation of the left labyrinth not the rhythmic quick responses in the nerve preceded by slow responses of the cerebellar cortex (C) Recovery (D) Immedi-

fications of the electrical activity of cerebellar cortex consisting either of tonic or of rhythmic responses

1 Tonic responses they were characterized by a long lasting activation of the cerebellar units which exhibited an increase in the discharge rate and recruitment of new units before silent, without signs of rhythm (Fig. 3 B)

2 Rhythmic responses they were characterized by the appearance of rhythmical outbursts of the electrical activity with intercalated periods of inhibition

In some cases the rhythmical responses consisted of sudden, asynchronous outbursts of a few units which lasted only 30-35 msec, each outburst being separated either from the preceding or from the following by a period of inhibition of the electrical activity which could last from 200 msec up to 1-2 sec. (Fig. 2 D) These rhythmical responses looked like the quick rhythmical responses recorded from the oculomotor nuclei (Azzena, 1966; Azzena & Girelli, 1967; Azzena *et al.*, 1967; Manni *et al.* 1965; Manni & Desole, 1966). In other cases the rhythmical outbursts were characterized by a longer duration (from 150 msec to two sec) and consisted of a progressive increase of the discharge rate with recruitment of other units. Short lasting (40 msec) periods of inhibition separated the single outbursts (Figs. 2 B F 3 C). Such type of response was similar to the slow responses of the oculomotor nuclei (Azzena, 1966; Azzena & Girelli, 1967; Azzena *et al.* 1967; Manni *et al.*, 1965; Manni *et al.* 1965; Manni & Desole, 1966). It is to be noted that no rhythmical responses were recorded from the other cerebellar lamellae and deep cerebellar structures.

#### *Analysis of the combination of the responses of the oculomotor nerve and of the cerebellum to the labyrinthine stimulation*

The cerebellar cortex and the oculomotor nerve exhibited various combinations of the above described responses to the labyrinthine stimulation. A tonic discharge could precede or follow the rhythmical outbursts either on the nerve or on the cerebellar cortex (Fig. 3 B). On the other hand a quick rhythmical response could be shifted to a slow one or vice versa by changing the side of stimulation. This occurred in about 1/3 of the rhythmical responses, in which the warm stimulation of a labyrinth induced quick responses of the ipsilateral cerebellar cortex while the stimulation of the contralateral labyrinth elicited slow responses (Fig. 2 B D). In the remaining 2/3 of the rhythmical responses only slow responses were observed

only after warm stimulation of the right labyrinth. The oculomotor nerve exhibited slow responses followed by quick outburst of the cerebellar cortex. (E, F) Guinea pig no. 478-E, normal activity: resting conditions F immersed into warm stimulation of the left labyrinth. The slow responses of the cerebellar cortex (arrows) began on the final part of the slow responses of the oculomotor nerve and continued during the quick inhibition of the nerve. Calibration: 100 msec.



On the other hand, if we take into account only the frequency of the rhythmical outbursts of the cerebellum and of the oculomotor nerve, the rhythmical responses can be collected in two different groups

*Group 1* This group was characterized by the fact that the rhythmical responses exhibited the same frequency on the cerebellum and on the oculomotor nerve. Some 81% of the recorded sites belonged to this group. In the majority of the cases both the cerebellum and the oculomotor nerve exhibited the same type of response for example, slow responses. On the other hand, in several experiments the nerve exhibited slow responses and the cerebellum quick ones or vice versa. However it is to be noted that the rhythmical outbursts exhibited by the cerebellum were not synchronous with those presented by the oculomotor nerve. In fact, the cerebellar outbursts were consistently delayed in relationship to the phases of the eye nystagmus. (Figs. 2B D F 3C)

*Group 2.* In the remaining 19% of the experiments the rhythmical cerebellar responses to the thermic stimulation of the labyrinth showed a frequency which differed from that presented by the rhythmical responses of the oculomotor nerve. The outbursts of the cerebellum were either more frequent or slower than those of the oculomotor nerve.

#### DISCUSSION

The result of the present investigation provided evidence that ampullar labyrinthine stimulation provoked the appearance of rhythmical responses on particular cerebellar areas.

Thus the conclusion may be reached that the cerebellum although not essential for the appearance of the eye nystagmus, is the seat of rhythmical activity when ocular nystagmus is present. Several investigators have pointed out that messages from muscles and joints arrive at the cerebellum (Dow & Moruzzi, 1938 Jansen & Brodal, 1954 Snider & Stowell, 1944) However the guinea pigs employed in this research were curarized and no proprioceptive impulses from the extra-ocular muscles during eye nystagmus could reach the brain-stem and the cerebellum. Thus it seems more reasonable to relate such rhythmical cerebellar activations to the ampullar receptors in the vestibular nuclei and the brain-stem reticular formation. It is a well known fact that other sensory organs (eye, hearing, skin) project on the cerebellum (Dow & Moruzzi, 1938 Jansen & Brodal, 1954 Snider & Stowell, 1944) The flocculus, paraflocculus and uvula were the cerebellar areas from which the rhythmical outbursts could be recorded. It is noticeable that such areas have clear-cut relationships to the vestibular apparatus (Brodal *et al* 1962) In fact they receive vestibular afferents and conversely they send fibers to the vestibular nuclei. However no rhythmical responses were recorded from the nodulus although it should be enclosed in the vestibular areas of the cerebellum. The absence of nodular rhythmical responses to labyrinthine stimulation supports the view of Jansen & Brodal

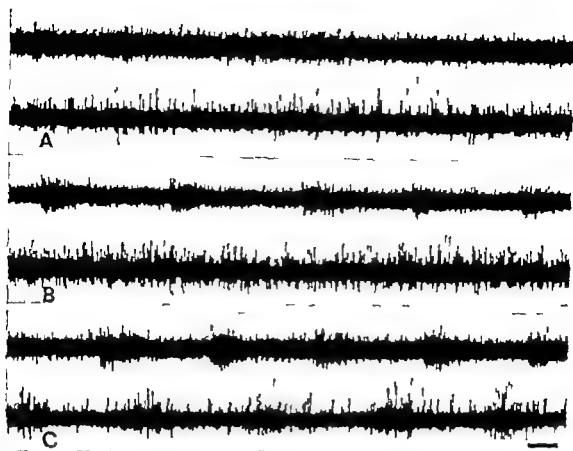


FIG. 2. Simultaneous records of the electrical activity of the left oculomotor nerve (upper beam) and of the unitary discharge of the right half of the n. ula (lower beam). Guinea pig n. 481. (A) Normal activity in resting conditions. (B) A warm stimulation of the left labyrinth induced rhythmical quick responses on the nerve (B-C) however at the beginning the cerebellar cortex presented tonic discharge (B) followed (C) by slow responses during the slow inhibition of the nerve. Calibration: 100 msec.

regardless of the stimulated side. A comparison of the response simultaneously recorded from the cerebellar cortex and from the oculomotor nerve offered some difficulty. In fact the responses may be differently grouped according to the various criteria of evaluation.

By taking into account only the type of response regardless of the stimulated and recorded sides the following combinations were observed:

1. In 74% of the stimulations both the cerebellar cortex and the oculomotor nerve exhibited rhythmical responses: the responses were slow in 20% and quick in 13% on both cerebellum and 3rd nerve, while in the remaining 32% the rhythmical outbursts were slow on the cerebellum and quick on the nerve or vice versa.

2. In 16% of the stimulations rhythmical responses occurred on the oculomotor nerve while the cerebellum presented tonic responses.

3. Finally in the remaining 10% of the stimulations the responses of both the cerebellum and oculomotor nerve were tonic.



et al 1958) Thus conclusion may be reached that the cerebellum utilizes messages from the vestibular apparatus in order to influence and regulate the correct performance of the extra-ocular movements just as it acts on the limb movements.

### ZUSAMMENFASSUNG

In den curarisierten Meerschweinchen wurde während des Augennystagmus, der durch die thermische Reizung des Labyrinths verursacht wird, mit Mikroelektroden die elektrische Aktivität der Kleinhirnrinde, gleichzeitig mit derjenigen weniger Fasern eines Nerves oculomotorius, aufgezeichnet. Der thermischen Reizung des Labyrinths folgend, wurden tonische und rhythmische Antworten der Uvula, des Flocculus und des Parafocculus aufgezeichnet. Die rhythmischen zerebellaren Antworten haben im 81% der Fälle die gleiche Frequenz des Augennystagmus ergeben. Die rhythmischen zerebellaren Antworten waren jedoch im Großteil der Fälle mit der langsamen oder schnellen Phase des Nystagmus nicht perfekt synchron. In den übrigen 19% der Fälle war die Frequenz der rhythmischen zerebellaren Antworten von der des Augennystagmus verschieden.

Es ergibt sich deshalb, dass das Kleinhirn, obwohl nicht wesentlich für das Erscheinen des Augennystagmus, Störungen der rhythmischen Aktivität während des Nystagmus ist. Auf diese Art benutzt das Kleinhirn Botschaften des Vestibularapparates, um die perfekte Ausführung der Augenbewegung zu beeinflussen, wie es für die Bewegung der Glieder handelt.

### REFERENCES

- ALLONCATTI, T. LEBENHITTE, F. RIVERO, D. A. GEMELLI, V. and LEROUX, N. 1948. Enregistrements de lographiques des mouvements oculaires dans le syndrome cérébelleux. *Rev. Neurol.* 94, 714.
- ARMSTRONG, S., and GRAY, T. B. E. 1934. Cortical projection of vestibular nerve in cat. *Acta Otolaryng.* (Stockh.) Suppl. 116 19.
- DAVIES, A. and PORTMAN, O. 1957. Microelectrode study of units of the rostral portion of the nucleus ambiguus. *Arch. Ital. Biol.* 95, 66.
- LEONARD, G., FAHLE, L. and GRAY, G. 1963. Nystagmus following stimulation of the central vestibular pathway using permanently implanted electrodes. *Acta Otolaryng.* (Stockh.) 5 pp. 192, 62.
- LEONARD, G. D. 1946. On inhibitory influences on the efferent discharge of the oculomotor nucleus. *Brain* 69, 2, 219.
- LEONARD, G. D. and B. METZ, M. L. 1947. Responses of the oculomotor units to deep cerebellar stimuli. *Brain* 70, 6, 323.
- LEONARD, G. D., GEMELLI, M. L. and DEBUI, P. L. 1947. Convergence of labyrinthine and cerebral vestibular impulses on brain-stem sites. *Exp. Neurol.* 19 401.
- RAUS, J. and LEONARD, G. 1952. Über den Einfluss der Ausschaltung verschiedener Hirnschichten auf die vestibulären Augenreflexe. *Arch. Neurol. Psychiat. Wien* 19 133.
- SHOUBAL, A., PORTMAN, O., and WALKER, F. 1957. The vestibular nuclei and their connections, anatomy and functional correlations. W. Ramsey Henderson, London.
- CARL, M. 1919. Le corrélat III des des du corrélat per excitation du labyrinthe. *Arch. Biol.* 1 62.
- 1921. La Fisiologia dell'apparato vestibolare. Zanichelli, Bologna. (Transl. R. J. Creed, 1930. Clarendon Press, Oxford.)

(1954) that a functional difference between flocculus and nodulus does exist

By recording simultaneously the action potentials of the cerebellar cortex and those of a few fibers of an oculomotor nerve it was possible to correlate the rhythmical cerebellar responses to the phases of the eye nystagmus elicited by the vestibular stimulation. Thus in the great majority of the cases (81%) the rhythmical outbursts recorded from the cerebellum exhibited the same frequency as that presented by the eye nystagmus. However only in a few cases were the rhythmical cerebellar responses perfectly synchronous with the phases of the eye nystagmus. frequently the cerebellar responses were slightly delayed in relationship to the quick or slow phases of the eye nystagmus. One could suppose that the impulses travelling from the vestibular nuclei and from the reticular bulbo-ponto-mesencephalic formation towards the cerebellum are mediated by chains of intercalated neurons. In fact Lorente de Nó (1933) and Horscholle & Tye Dumont (1968) pointed out that the mechanism of the eye nystagmus involves several chains of neurons in the brain stem. It is more difficult to find an explanation for the cases in which the cerebellar responses exhibited a clear-cut different frequency from that of the eye nystagmus. Recently also Manni & Girelli (1968) observed that the rhythmical outbursts exhibited by the vestibular nuclei after labyrinthine stimulation were not synchronous with the phases of the eye nystagmus. In some instances the vestibular nuclei showed a higher frequency than the eye nystagmus. By taking into account the connections of vestibular nuclei with the cerebellar areas one could suppose that such types of rhythmical cerebellar responses were driven by the vestibular nuclei.

It is to be noted that the rhythmical responses of the cerebellum to the labyrinthine stimulation escaped the attention of the other researchers who analyzed the electrical activity of the cerebellum during labyrinthine stimulation in mammals. In fact only tonic cerebellar responses were observed (Arduini & Pompeiano 1957, Camis, 1919, Price & Spiegel 1937, Riva Sansaverino & Urbano, 1965, Weber & Steiner 1965). Only Gualtierotti et al (1958, 1959) pointed out the relationships of cerebellar rhythmical outbursts to the nystagmus of the pigeon head during and after angular accelerations of the animal. However no rhythmical responses were reported in the experiments carried out by Gualtierotti & Passerini (1958) in the cat. they related the cerebellar electrical activity of the cat to the action potentials of the neck muscles and they did not take into account the electrical activities of the extra-ocular muscles or of the oculomotor nerve.

Now a question arises: what is the functional meaning of such cerebellar rhythmical activity in relationship to the eye nystagmus. It is to be noted that alterations of the extra-ocular movements were observed in human beings with cerebellar diseases as well as in totally cerebellectomized animals: there was delay and slow contraction, hypermetria, oscillation in the movements and in maintaining the position of the eyes (Alajouanine

- HOOGMOED, G., and T. C. DEMONT, B., 1948: Acti 116a unitaire des neurones vestibulaires et oculomoteurs a regard du nystagmus. *Exp. Brain Res.* 5, 16.
- BOGNER, T. 1931: Beiträge zur Funktion des Kleinhirns beim Kanarienvogel. *Arch. Otolaryng. (Stech.)* Suppl. 2, 1.
- JAMES, J. and BOGNER, A., 1931: A text of cerebellar anatomy. Johann Grunig's Verlag, Oulu.
- KOLLE, W. 1933: Motor effects from electrical stimulation of human cerebellum in the unanesthetized cat. *J. Neurophysiol.* 18, 559.
- LOEWYER DE M., R., 1931: A graphisch Kapitel aus der vergleichenden Physiologie des Labyrinthes. Die Augenmuskelflexion beim Kanarienvogel und ihre Grundgesetze. *Ergebn. Physiol.* 22, 73.
- 1933: Vestibulo-ocular reflex arc. *Arch. Neurol. Psychiat.* 30, 344.
- DE GUER, CLAUDE, W. R., 1936: The termination of ascending tract in the thalamus of the macaque monkey. *J. Anat.* 71, 7.
- LOEWYER DE M., R., 1916: Über die Anatomie und Physiologie der Formica terminalis cerebelli. *Arch. Neurol. Psychiat.* 21, 349.
- MARCA, R., 1914: Welche Teile des Zentralnervensystems müssen für das Zustandekommen der tonischen Hals- und Labyrinthreflexe auf die Körpermuskulatur vorhanden sein? *Arch. Ges. Physiol.* 140, 231.
- MARCA, R., W. MARX, W. R., and MARXON, E. W., 1923: Electrical stimulation of the interior of the cerebellum in the monkey. *Amer. J. Physiol.* 112, 229.
- MARCA, R., 1926: Localizzazione cerebellari corticali nella ca. II. Effetti di lesioni delle parti embolari del cervelletto. *Arch. Fisiol.* 60, 119.
- 1946: Contributi delle registrazioni microelelettrode extracellulari alla fisiologia di alcune strutture bulbo-pontino-mesencefaliche. In *Conferenze di aggiornamento I della Società Italiana di Elettroneurologia e Neurofisiologia*. Auto Gaggi, Bologna.
- MARCA, R., AZZURA, G. B., CASATI, H., and DOW, R. E., 1945: Influence of the labyrinth on the unitary discharge of the oculomotor nucleus and some adjacent formations. *Exp. Neurol.* 12, 2.
- MARCA, R., AZZURA, G. B., and DOW, R. E., 1946: Cerebellar influences on the unitary discharge of the oculomotor nucleus and adjacent structures. *Exp. Neurol.* 12, 352.
- MARCA, R., and DOW, R. E., 1946: Responses of oculomotor units to the stimulation of single semicircular canals. *Exp. Neurol.* 13, 306.
- MA, T. E., and GERTZ, M. L., 1949: Vestibular nuclei influenced by labyrinthine and cerebral ymaginogenic impulses. *Exp. Neurol.* 22, 143.
- MARCA, F. R., and LAZZARONI, R. E., 1938: The function of cerebellar nuclei as determined by electrical stimulation. *Arch. Neurol. Psychiat.* 29, 47.
- MARCA, F. R., 1944: Note osservazioni intorno gli effetti della stimolazione del cervelletto. *Atti del 1° Congresso Nazionale della Società Italiana di Neurofisiologia e Neurologia*. Boll. Soc. Ital. Biol. Sper. 24, 712.
- PROSS, J. B., and BRIDGES, R. A., 1927: Vestibulo-cerebral pathway: A contribution to the central mechanism of nystagmus. *Arch. Otolaryng. (Chic.)* 26, 432.
- RAJAN, A. T., 1923: Origin and course of the fasciculus medialis (Rexed) in the cat. It is shown that an other fibre tract arising from the cerebellar nuclei. *J. Comp. Neurol.* 6, 183.
- RAJAN, A. T., and LAMARCA, A., 1945: Electrical activity of paraflocculus and other cerebellar lobules during of vestibular rotatory stimulation in the cat. *Arch. Sci. Biol.* 13, 23.
- SCHUBERT, A., and DUMAS, J. M. R., 1938: Cerebro-cerebellar relations in the cat. *J. Neurophysiol.* 21, 1.
- SIMONELLI, G., and DE GIAMBERI, A. M., 1933: Il comportamento del nistagmo pontano provocato nelle lesioni anatomiche del cervelletto. *Boll. Soc. Ital. Biol. Sper.* 2, 204.
- STONER, R. E., and STONER, A., 1944: Neural lag areas of the tactile, auditory and visual systems in the cerebellum. *J. Neurophysiol.* 7, 321.

- CARPENTER, M. B., and STROMINGER, N. L., 1964: Cerebello-oculomotor fibers in the Rhesus monkey *J Comp Neurol*, 123 211.
- CHAMBERS, W. W. Jr 1947: Electrical stimulation of the inferior cerebellum in the cat. *Amer J Anat* 30 55.
- CHAMBERS, W. W. and SPRAGUE, J. M. 1955: Functional localization in the cerebellum. II. Somatotopic organization in cortex and nuclei. *J. Neurophysiol* 18 633.
- CHANG, H. T. and KOSTYUK, P. G. 1960 Discharge of single neurons of the toad cerebellum evoked by stimulating the vestibular nerve *Fiziol Zh. SSSR Sech nov* 46 926 Quoted by *Excerpta Med* 11 1961.
- CHATELIER, G. and LETTNER, L. M., 1963: Localisation d'unités synchrone du nystagmus dans le rhombencéphale du lapin. *J Physiol (Par)* 54 312.
- COMCK, H. GORU, K., SHAMBER, S. and WEISS, A. H. 1965 Eye movements induced by electrical stimulation of the cerebellum in the alert cat. *Exp Neurol* 13 145.
- DE KLEUK, A. and MAGNUS, R., 1920 Über die Unabhängigkeit der Labyrinthreflexe vom Kleinhirn und über die Lage der Zentren für die Labyrinthreflexe im Hirnstamm. *Arch Ges. Physiol*, 178 124.
- DI GIORGIO, A. M. 1950 Comportamento delle reazioni oculari labirintiche nelle lesioni di una metà laterale del cervelletto *Boll. Soc. Ital Biol Sper* 26 31.
- DOW, R. S. 1938 Effects of lesions in the vestibular part of the cerebellum in primates. *Arch Neurol Psychiat* 40 500.
- 1939: Cerebellar action potentials in response to stimulation of various afferent connections. *J Neurophysiol* 2 543.
- DOW, R. S. and MARSH, E., 1964: The relationships of the cerebellum to extra-ocular movements. In *The oculomotor system* Harper & Row New York.
- DOW, R. S., and MORRIS, C. 1958 *The physiology and pathology of the cerebellum* University of Minnesota Press, Minneapolis.
- DÜRSING, F. and SCHARFVER, K. P. 1957: Die Arouenessaktivität in der Formatio reticularis des Rhombencephalons beim vestibulären Nystagmus. *Arch Psychiat* 196 263.
- 1957: Die „Locke gekoppelten“ Arouness der Formatio reticularis des Rhombencephalons beim vestibulären Nystagmus. *Arch Psychiat* 196 402.
- DUMONT TYG, S., and DELL, P. 1961 Analyse électrophysiologique de l'arc réflexe vestibulo-oculaire *J Physiol (Par)* 53 316.
- FERNÁNDEZ, C., and FERNÁNDEZ, J. M. 1963 Experimental cerebellar lesions and their effects on vestibular function *Acta Otolaryng (Stockh.) Suppl.* 192 52.
- FERRARO, A. and BARRERA, S. E., 1936 Effects of lesions of the juxtarestiform body (I.A.K. bundle) in Macaca hesus monkey *Arch Neurol Psychiat* 35 13.
- GERMANN, B. E., 1964 Vestibular connections in the brain stem. In *The oculomotor system* Harper & Row New York.
- GRANT, G. ASCHAN, G. and RAY, L. L., 1963: Nystagmus produced by localized cerebellar lesions. *Acta Otolaryng (Stockh.) Suppl.* 192 78.
- GU LTIENOTTI, T. and PASARINI, D. 1958 Soglia cerebellare all'accelerazione rotatoria somministrazione per la pelle di gatti. *Atti Acc. Lincei* VIII 25 115.
- 1958 Risposta rotatoria postrotatoria cerebellare a variazioni di pressione statica e dinamica. *Atti Acc. Lincei* VIII 25 584.
- GUALTIEROTTI, T., SCHREIBER, B., MANN, L. H. and PASARINI, D. 1959 Effect of acceleration in cerebellar potentials in bird and its relationship to direct stimulation. *J Physiol* 197 489.
- HARRIS, R. 1950 Eccitamento globale del labirinto e lesioni limitate della corteccia cerebellare *Boll. Soc. Ital Biol Sper* 26 43.
- HARRIS, W. K., MAGDOUX, H. W. and RANSON, S. W. 1936 Electrical stimulation of the inferior part of the cerebellum in the decerebrate cat. *Amer J Physiol* 117 251.
- 1937: Localization within the cerebellum of responses to focal cerebellar stimulation. *J Comp. Neurol* 67 145.

## ACTIVE ION TRANSPORT FROM THE SCALA VESTIBULI INTO THE SCALA MEDIA

### *Participation therein of Reissner's Membrane and its Blockade*

J. PRAŽMA

*From the Laboratory for Otolaryngology Czechoslovak Academy of Sciences  
Prague Czechoslovakia*

The effect of G-strophantidin and 2,4-dinitrophenol introduced into the scala vestibuli of 24 guinea pigs was studied by measuring endocochlear and microphonic potentials (EP and MP) which indicate ion transport and the activity of hair cells. MP were evoked by tones of 500 1000 and 2000 c/sec. and an intensity of 60 dB. MP were always found to change together with EP but in dependence on changes of EP. Inhibition of ion transport with G-strophantidin introduced into the scala vestibuli decreased EP to nearly zero while 2,4-dinitrophenol even caused EP to show negative values which in part represent the diffusion potential of certain ionic difference between the cochlear duct and the surroundings. It is concluded that not only the stria vascularis, but also Reissner's membrane, participate in active ion transport between the cochlear duct and its surroundings. The active ion transport is a source of EP.

In recent years increased attention has been paid to electrophysiological and biochemical changes in the cochlear duct and to the origin of the endolymph. Békésy in 1952 described endocochlear potentials (EP) in the cochlear ductus between +60 and +80 mV. Smith *et al* (1954) demonstrated that the composition of the endolymph differed from that of other extracellular fluids. In the guinea pig it has a high level of K<sup>+</sup> (144 meq/l) and a low level of Na<sup>+</sup> (15.8 meq/l) while the composition of the perilymph is the opposite (140 meq/l Na<sup>+</sup> and 4.8 meq/l K<sup>+</sup>). Similar values were later obtained for man (Citron *et al* 1956 Rauch & Kostlin, 1958 Hladký & Opletal, 1965). Smith *et al* (1958) found the K<sup>+</sup> and Na<sup>+</sup> concentration to be the same in the whole endolymphatic system although the EP have different values in different parts of the membranaceous labyrinth: cochlear duct +80 mV, sacculus -1 mV, utricle +4 mV and ampulla -1 mV and this indicates that EP values do not depend on the concentrations of salts in different parts of the labyrinth.

Tasaki *et al* (1954) Davis (1957) Tasaki & Spyropoulos (1959) and Vosteen (1961) believe that the stria vascularis is the source of EP and consider Reissner's membrane to be an effective insulator. Rauch (1962,

- SPINELLI, E. A., and SCALA, N. P. 1941: Vertical nystagmus following lesions of cerebellar vermis. *Arch. Ophthalmol* 26 861
- 1942: Positional nystagmus in cerebellar lesions. *J Neurophysiol* 5, 247
- WHITTSIDE, J. A. and SWINER, R. S., 1953: Relation of the cerebellum to upper brain stem. *J Neurophysiol* 16 397
- VON WEER, O. and STIEGER, F. A., 1965: Labyrinthär erregbare Neurone im Hirnstamm und Kleinhirn der Ratte. *Helv Physiol Acta*, 22 61

*Institute of Human Physiology University  
of Sassari Via Murroni 23,  
07100 Sassari, Italy*

*Received August 5, 1968*

## ACTIVE ION TRANSPORT FROM THE SCALA VESTIBULI INTO THE SCALA MEDIA

### *Participation therein of Reissner's Membrane and its Blockade*

J. PRAŽMA

*From the Laboratory for Otolaryngology Czechoslovak Academy of Sciences  
Prague, Czechoslovakia*

The effect of G-strophantin and 2,4-dinitrophenol introduced into the scala vestibuli of 24 guinea pigs was studied by measuring endocochlear and microphonic potentials (EP and MP) which indicate ion transport and the activity of hair cells. MP were evoked by tones of 500 1000 and 2000 c/sec. and an intensity of 80 dB. MP were always found to change together with EP but in dependence on changes of EP. Inhibition of ion transport with G-strophantin introduced into the scala vestibuli decreased EP to nearly zero while 2,4-dinitrophenol even caused EP to show negative values which in part represent the diffusion potential of a certain ionic difference between the cochlear duct and the surroundings. It is concluded that not only the stria vascularis, but also Reissner's membrane, participate in active ion transport between the cochlear duct and its surroundings. The active ion transport is a source of EP.

In recent years increased attention has been paid to electrophysiological and biochemical changes in the cochlear duct and to the origin of the endolymph. Békésy in 1952 described endocochlear potentials (EP) in the cochlear ducts between +60 and +80 mV. Smith *et al* (1954) demonstrated that the composition of the endolymph differed from that of other extracellular fluids. In the guinea pig it has a high level of K (144 meq/l) and a low level of Na (15.8 meq/l) while the composition of the perilymph is the opposite (150 meq/l Na and 4.8 meq/l K). Similar values were later obtained for man (Citron *et al* 1956 Rauch & Hostlin, 1958 Hladky & Opletal, 1963). Smith *et al* (1958) found the K and Na concentration to be the same in the whole endolymphatic system although the EP have different values in different parts of the membranaceous labyrinth: cochlear duct +80 mV, sacculus -1 mV, utricle +4 mV and ampulla -1 mV and this indicates that EP values do not depend on the concentrations of salts in different parts of the labyrinth.

Tanaka *et al* (1934) Davis (1937) Tanaka & Spyropoulos (1959) and Vosteen (1961) believe that the stria vascularis is the source of EP and consider Reissner's membrane to be an effective insulator. Rauch (1962,

1963, 1966) on the other hand, and Choo & Tabowitz (1964) demonstrated rapid transfer of  $^{24}\text{Na}$  and  $^{42}\text{K}$  from the scala vestibuli into the scala media.  $\text{K}$  was found to be transported three times as rapidly as  $\text{Na}$  from the perilymph into the endolymph although  $\text{K}$  is transported against a greater concentration gradient. Active ion transport by cells and the origin of membrane potentials have been studied extensively (Ussing, 1948 Harris & Maizels, 1951 Steinbach, 1952 Hodgkin & Keynes, 1955 Janáček, 1963)

Substances inhibiting active transport include G-strophantidin and 2,4-dinitrophenol Schatzmann (1953) explains the inhibitory effect of G-strophantidin by a direct action on transport activity without there being an effect on total cell energy metabolism The blocking effect of 2,4-dinitrophenol is due to inhibition of ATP formation which is the energy source for active ion transport.

These findings led us to inquire how far EP depend on the activity of cells of Reissner's membrane This touches problems of the origin of the endolymph, metabolism in the inner ear and the origin of cochlear potentials

#### METHOD

A total of 24 hearing guinea pigs weighing 250–300 g was anaesthetized with 0.8 ml/100 g body weight 20% urethane. EP and microphonic potentials (MP) were recorded after opening the bulla on the ventral side and introducing glass microelectrodes with a micromanipulator through the stria vascularis into the scala media in the 2nd coil Glass microelectrodes with a resistance of 4–7 M ohm and were filled with 3 M KCL connected via a chlorinated Ag wire across a cathode-ray follower to a direct-current amplifier Amplified cochlear potentials were recorded with an ink recorder and controlled on an oscillograph

For MP determinations a tone of 500 1000 and 2000 c/sec and 60 dB intensity was applied for one sec. The intensity of the sound was approximately determined at the entry into the meatus with an artificial recording ear

For perfusions the scala vestibuli was opened at the basilar coil and at the top of the labyrinth with a fine drill Into the basilar coil a fine cannula was introduced which led the perfusion fluid into the scala vestibuli. The fluid left the apex for the bulla and was there collected with cotton wool tampons (Fig. 1) The rate of perfusion was such that the pressure of the fluid did not cause changes in the endocochlear potentials. In group I (control) only Ringer's solution was used, in group II  $8.5 \cdot 10^{-3}$  M G-strophantidin in Ringer's solution and in group III  $1 \cdot 10^{-3}$  M 2,4-dinitrophenol previously neutralized also in Ringer's solution

At the start of the experiment lasting 60 min, initial values of EP and MP were determined. In the 5th min the perfusion cannula was introduced Between the 10th and 15th min the scala vestibuli was perfused with the chosen solution All EP and MP values were continuously recorded For



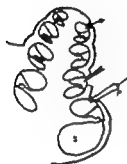


Fig. 1

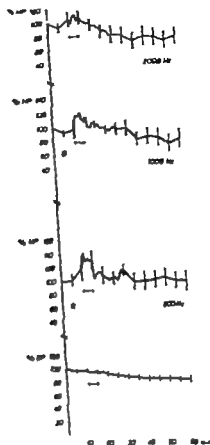


Fig. 2

Fig. 1 Schema of perfusion of the scala vestibuli. S.T. scala tympani; S.V. scala vestibuli; P canal, inserted; S.V. microelectrode inserted in scala media; II the 2nd cochlear coil

Fig. 2 Effect of perfusion of the scala vestibuli with Ringer's solution on EP and MP. A, MP response in % to tone at 2000/sec; B, the same at 1000/sec; C, the same at 500/sec; II endocochlear potentials in % to time of perfusion of scala vestibuli; I, D

statistical evaluation. Values found at 5-minute intervals, and between the 10th and 45th minute one-minute intervals were used. Because of the scatter of values all results had to be calculated as percentages so that they could be compared—except EP in group III. The mean value from determinations at the start of the experiment after opening the scala vestibuli was taken as 100.

In group III EP values fell below zero after 2,4-dinitrophenol application and thus their negative value was due in addition to the generated positive EP also to the diffusion potential which is negative and due to the difference in the concentration of ions in the scala media and its surroundings. Here absolute values were used to express the relationship more exactly.

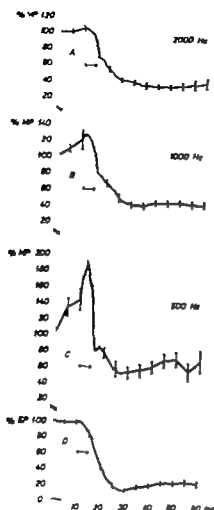


FIG. 3

FIG. 3 Effect of perfusion on scala vestibuli with *G. atropa tin* on EP and MP and MP response in % to tone of 2000 c/sec A the same to 1000 c/sec B, the same to 500 c/sec C, the same to 500 c/sec D endocochlear potentials in %  $\leftrightarrow$  time of perfusion of scala vestibuli  $\downarrow$  a.s.

FIG. 4 Effect of perfusion on scala vestibuli with 2,4-dinitrophenol on MP and EP and MP response in % to tone of 2000 c/sec A the same to 1000 c/sec B, the same to 500 c/sec C, the same to 500 c/sec D endocochlear potential in %  $\leftrightarrow$  time of perfusion of scala vestibuli  $\downarrow$  a.s.

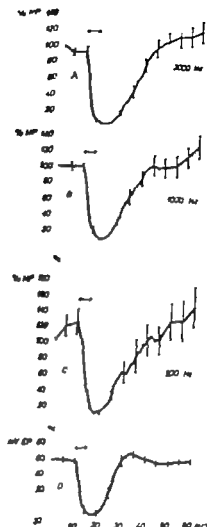


FIG. 4

All data were evaluated statistically at the given time intervals. Figures were prepared from the arithmetic means and standard deviations. The student's *t* test was used.

## RESULTS

In group I (control—Fig. 2 A B C D) the scala vestibuli was perfused from the 10th to the 15th minute. Perfusion did not cause changes in the EP (Fig. 2 D). In the 4th coil (corresponding to tones of 500 c/sec) MP values were significantly increased during perfusion (Fig. 2 C). In coil 2 and 3 (tones 1000 and 2000 c/sec) no effect was seen (Fig. 2 B A).

In group II perfusion with G-strophanthin caused a fall in EP values, significant from the 6th minute after the start of perfusion to the end of the experiment. The largest decrease (to  $11\% \pm 2.04$ ) was found in the 30th minute (Fig. 3 D). The initial rise in MP values was due to application of the cannula to the opening of the basilar coil of the scala vestibuli before perfusion. This factor affected particularly the frequency in the 4th coil (500 c/sec, Fig. 3 C) and partly also in the 3rd coil (1000 c/sec Fig. 3 B). Within 3 min after the start of perfusion, MP increased slightly and this was significant particularly in coil 4 (500 c/sec). Then they started to fall so that there was a statistically significant decrease between the 6th minute of perfusion and the end of the experiment. The lowest MP values were found in the 28th minute of the experiment (Fig. 3 A B C).

In group III (Fig. 4 A B C D) perfusion with 2,4-dinitrophenol caused a pronounced fall in EP which was significantly different from group I in the 18th minute of the experiment, i.e. 3 min after start of perfusion and which fell to negative values in the 14th minute (Fig. 4 D).

The largest fall in EP to  $-13.6 \text{ mV} \pm 1.02$  was found in 18th minute. Then EP started to rise again, so that in the 31st minute initial values were again obtained. The MP values fell in the 2nd, 3rd and 4th coil at the same time (Fig. 4 A B C). The largest fall was again in the 18th minute where MP attained zero values, to 10% of the average initial values. Since the effect of 2,4-dinitrophenol is short-lasting, MP started to rise after the 18th minute and reached initial values in the 40th minute.

Thus both G-strophanthin and 2,4-dinitrophenol caused a fall in EP and MP while Ringer's solution itself had no such effect.

G-strophanthin maintained decreased EP up to the end of the experiment but zero values were not attained. 2,4-dinitrophenol, on the other hand, caused a rapid fall in EP down to negative values. Its effect was short lasting and hence in the 31st minute values returned to normal.

MP values were always found to alter together with EP values. It may be concluded that Reissner's membrane which separates the space of the scala vestibuli from the scala media is not completely impermeable to ions and that its cells participate in the ionic exchange between these spaces.

#### DISCUSSION

N. Stalin & Harrison (1958) first applied the finding of Hodgkin & Keynes (1955) on membrane potential to the inner ear. They put forward the hypothesis that ions pass through the Reissner's membrane. Rauch & Kostlin (1962, 1963) and Choo & Tabowitz (1964) showed that labelled Na and K pass through this membrane and this is also indicated by electronmicroscopic work. Lawrence *et al* (1961) Iurato (1967) Duval (1967) showed that the layer of epithelial cells of the Reissner's membrane facing the ductus cochlearis contain many mitochondria and other particles. The inner surface of these cells has many microvilli. Adenosine triphosphatase activity

has also been demonstrated on the surface of the endolymphatic area of the membrane and this enzyme is found at sites of active transport Nakai & Hilding (1967) Using's work (1960) and that of others shows that active ionic transport across membranes depends on respiration or glycolysis (mammalian red blood cells) since inhibition of these processes or anoxia of the whole animal leads to a fall in ion transport Reissner's membrane also behaved thus in decapitated animals (Rauch) Inhibition with G-strophantidin, however of ionic transport has no effect on oxygen consumption and glycolysis (Schatzmann, 1953) but influences ion transport mechanism.

It must be considered whether G-strophantidin and 2,4-dinitrophenol can not block ionic transport from the perilymph into endolymph across the tissue of the lateral wall, i.e. via the spiral ligament into the stria vascularis. Tonndorf *et al* (1962) investigated the propagation of various dyes after their application into the scala vestibuli and they found that the Reissner's membrane is relatively impermeable. It is therefore assumed that ions are transported from the scala vestibuli into the scala media via the spiral ligament and the stria vascularis.

It is well known that 2,4-dinitrophenol and G-strophantidin block the active ion transport. After the application of these substances into the scala vestibuli the EP decreased almost to zero which proves that EP is generated by the active ion transport According to Tasaki & Spyropoulos (1959) the stria vascularis represents the source of EP whereas the findings of Lawrence *et al* (1961) Duval (1967) Nakai & Hilding (1967) and Iurata (1967) support our view that the Reissner's membrane also partly participates in the active ion transport

Davis *et al* (1955) found that the EP depend more on oxidative mechanisms than on the ionic difference in the surroundings medium This does not contradict our findings. The active ionic transport described here is dependent on aerobic or anaerobic processes. Davis (1957) considers the wall of the cochlear duct to be practically impermeable to ions This conclusion is based on the work of Békésy (1951) who found that the wall of ductus cochlearis is very resistant to the direct current and to the low frequencies of alternating current Rauch (1964) on the other hand, states that no membrane of the living cells with a relatively high metabolic rate is impermeable or nearly impermeable to water and ions and also demonstrates this. Chou (1963) showed by respirometry that Reissner's membrane has the same rate of oxygen consumption as the stria vascularis and values are among the highest found in the animal Honrubia *et al* (1965) showed that perfusion of the scala vestibuli in anoxaemic guinea pigs maintains the EP at normal values while perfusion of the scala tympani has no effect on the fall in EP due to anoxia Thus again Reissner's membrane seems to be permeable in these experiments.

Our data on the rapid inhibition of EP and MP by the drugs used agree with those of Rauch *et al* on ion transport across Reissner's membrane and in addition show that EP are generated by active transport of ions.

## ZUSAMMENFASSUNG

Bei 24 Meerschweinchen wurde der Einfluss von G-Strophantidin und 2,4-Dinitrophenol nach deren Applikation in die Scala vestibuli auf den aktiven Ionentransport durch Messung der endocochleären und mikrophonen Potentiale (EP und MP) ermittelt. Die MP wurden mittels Tönen von 500, 1000 und 2000 Hz bei einer Intensität von 60 dB untersucht. In allen Versuchen wurde beobachtet, dass die MP immer parallel in Abhängigkeit von den Veränderungen der EP sinken oder ansteigen.

Es wurde festgestellt, dass die Blockade des Ionentransportes durch die Applikation von G-Strophantidin in die Scala vestibuli einen Rückgang des EP beinahe bis auf einen Nullwert verursacht. Die Applikation von 2,4-Dinitrophenol in die Scala vestibuli hatte sogar einen Rückgang der positiven EP Werte bis zu negativen Werten zur Folge, wo die Messwerte einen Teil des diffusen, durch die Ionendifferenz zwischen dem Cochlear duct und der Umgebung bestimmten Potentials darstellen.

Aus diesen Ergebnissen wird darauf geurteilt, dass an dem Ionenaustausch zwischen dem Ductus cochlearis und der Umgebung nicht nur die Stria vascularis, wie bereits früher nachgewiesen wurde sondern auch die Reissnermembran beteiligt ist. Der aktive Ionentransport bildet eine Quelle des EP.

## REFERENCES

- BASTY, G. O., 1951 The coarse pattern of the electrical resistance of the cochlea of the guinea pig (electro-anatomy of the cochlea). *J. Acoust. Soc. Amer.* 23, 18.
- 1952 DC resting potentials and the cochlear partition. *J. Acoust. Soc. Amer.* 24, 72.
- CHAO, Y. B. and TANOWITZ, B. 1961 The formation and flow of the cochlear fluid. I. Studies with radioactive sodium (Na). *Ann. Otol.* 73, 87.
- 1963 II. Studies with radioactive potassium (K). *Ann. Otol.* 74, 140.
- CHAO, L. T. Y. 1963 Respiration of Reissner's membrane of the guinea pig. *J. Laryng.* 77, 374.
- DANIEL, H. 1937 Biophysics and physiology of the inner ear. *Phys. Rev.* 57, 1.
- DANIEL, H., TASHAK, L., SWARTZ, C. A. and DEAN MERRICK, B. H., 1936 Cochlear potentials after intracochlear injections and oxia. *Fed. Proc.* 15, 112.
- DANIEL, H. and RICHMOND, V. T. 1967 Reissner's membrane. *Arch. Otolaryng. (Chic.)* 88, 143.
- HARRIS, E. J. and MARRAS, M. 1931 The permeability of human red cells to sodium. *J. Physiol. (Lond.)* 113, 306.
- HLADIK, R. and OPEKAL, A., 1963 Průchodnost buněk elektrolytů a glukózy perilymfy chlojky pokusného zvířete. *Otolaryng. Česl.* 14, 192.
- HODGKIN, A. L. and KATZ, R. D., 1955 Active transport of calcium in giant axons from *Helix*. *J. Physiol. (Lond.)* 122, 29.
- HODGKIN, A. L., JON STOKES, B. M. and BATES, R. A., 1965 Metal ionic effects on cochlear potentials. *J. Physiol. (Lond.)* 122, 105.
- KATO, S., 1962 Submicroscopic structure of the membranous labyrinth. III. The peripheral structure of Corti's organ (Basilar membrane, limbus piratus and pirus). *J. Electronmicroscopy*, 11, 40.
- 1967 Elektronenmikroskopische Struktur der Innenohr-Membranen mit Rückschlüssen auf ihre Funktion am Stoffwechsel. *Arch. Klin. Exp. Ohr Nas. Kehlkopfheilk.* 123, 112.
- JOHNSON, K., 1963 A transient electrophysiological phenomenon in frog skin. *Physiol. Behav.* 12, 249.

- LAWRENCE, M WOLSK, H and LITTON, W B 1961 Circulation of the inner ear fluids. *Ann Otol* 70 753
- NAFTALIN, L., and HARRISON M. S 1958 Circulation of labyrinthine fluids. *J Laryng* 72 118.
- RAUCH, S 1962 Biochemische Studien zum Hörvorgang Zur Funktion d = Reissner'schen Membran *Z Laryng Rhinol Otol* 41 56
- 1963: Biochemische Studien zum Hörvorgang Die resorptive Funktion der Stria vascularis *Prakt Otorhinolaryng* (Basel) 25 81
- 1964 *Biochemie des Hörorgans*. Georg Thieme, Stuttgart
- 1966 Membrane problems of the inner ear and their significance *J Laryng* 80 11 44
- RAUCH, S and KÖSTLIN, A 1958: Aspects chimiques de l'endolymphe et de la perilymphe. *Pract Otorhinolaryng* (Basel) 20 287
- RAUCH, S KÖSTLIN A SCHNEIDER, E. A and SCHINDLER, K., 1963 Arguments for the permeability of Reissner's membrane. *Laryngoscope* 73 135
- SCHATZMANN J H 1953: Herzglykosid als Hemmstoffe für den aktiven Natrium und Natrium Transport durch die Erythrocyt membran. *Helv Physiol Pharmacol Acta*, 11 346
- SMITH, C. A DAVIS, H DEATHERAGE, B. H and GRASSET C. F 1958: DC potential of the membranous labyrinth *Am. J Physiol* 193 203
- SMITH, C. A., LOWRY O H., and WU M. L., 1954: The electrolytes of the labyrinthine fluids. *Laryngoscope* 64 141
- STEINBACH, H B 1952: On the sodium and potassium balance of isolated frog muscles. *Proc Nat Acad Sci U.S.A* 38 451 Cit. Ussing 1960
- TABAKI, I DAVIS, H and ELDERIDGE, D H 1954 Exploration of cochlear potentials in guinea pigs with a microelectrode *J Acoust Soc. Am* 26 65.
- TABAKI, I and SAKOPOLLOS, C. S 1959: Stria vascularis as source of endocochlear potential *J Neurophysiol* 22 149
- TOMNDOFF I DUVALL, A J and RENEAU I P 1962 Permeability of intracochlear membranes to various vital stains. *Ann Otol* 71 801
- USSING, H H 1948: The use of tracers in the study of active ion transport across animal membranes. *Cold Spring Harbor Symposia Quant Biol* 13 193
- 1960 The alkali metal ions in biology. In *Handbuch der experimentellen Pharmakologie* Springer Berlin, Göttingen, and Heidelberg.
- VOSTERN K. H 1961 Versuch Aspekt zu Biologie und Pathologie des Innenohres *Arch Ohr Nas Kehlkopfheilk* 178 1

Lab für Otoryngology

Czechoslovak Academy of Science

Prague 2 Czechoslovakia

Received May 14 1968

# DAS FREQUENZMAXIMUM DES KALORISCH AUSGELOSTEN NYSTAGMUS I ALS KENNLINIENFUNKTION DES GEPRÜFTEN VESTIBULARORGANES

C. CLAUSSEN

*Aus der Hals-Nasen-Ohren-Klinik der Freien Universität, Berlin  
Deutschland*

Die Schlagfrequenz des postkalorisch  $\alpha$  Nystagmus I, welche aussagekräftiger ist als die Dauer wird in dieser Arbeit zur Beurteilung des geprüften Vestibularorganes dergestalt benutzt, daß aus der Schlagrate während des 30-Sekunden Bereiches maximaler Schlagfrequenz ein Kennlinienzeiger abgeleitet wird. Bei entsprechender Anordnung der vier Kennlinien der Warm- und Kaltprüfung beider Ohren in den vier Quadranten eines rechtwinkligen Koordinatensystemes kommt man zu einer Zeigerdarstellung, die eine vereinfachte Beurteilung der Ergebnisse der kalorischen Vestibularisprüfung ohne ENG und PENG zuläßt. Durch entsprechende Konstellationen der Kennlinienzeiger erhält man einprägsame typische Muster für z. B. periphere vestibuläre Seitendifferenzen, zystisches Richtungsüberwiegen, Spontanystagmus, Verlaufskontrolle etc. Die Anwendbarkeit dieser Methode wurde bei 126 kalorischen Vestibularisprüfungen erprobt.

Diese Arbeit will versuchen, dem praktisch tätigen Otoneurologen auch ohne Elektronystagmographie und Photoelektronystagmographie allein mit der Frenzel Brille und einer Stoppuhr oder einem einfachen Zeitkurvenschreiber eine Möglichkeit an die Hand zu geben, durch eine entsprechende Auswahl und Anordnung seiner Beobachtungen relativ schnell zu einer quantitativen Aussage über den postkalorischen Nystagmus I zu kommen, die an Aussagekraft das bisher übliche Schema von Halpike (Fitzgerald & Halpike, 1942) übertrifft, welches die Nystagmusedauer als Grundlage benutzt.

Ein Parameter der Vestibularisfunktion, der für jeden einzelnen kalorischen Reiz auch eine Reizantwort nach Art einer Häufigkeitsverteilung ähnlich der Normalverteilung nach Gauss liefert (Hinrichs, 1967; Torok, 1967) ist die Frequenzverteilung des postkalorischen Nystagmus I. Das Nystagmusfrequenzmaximum ist besonders leicht beobachtbar, da sich zu diesem Zeitpunkt auch die Amplitude im Maximalbereich befindet. Das Integral der Frequenzverteilung über der Zeit ergibt eine kumulative Verteilung, die aus der Technik als sog. Kennlinie eines geprüften Prozesses oder eines gesteuerten Systems bekannt ist. Zum Ablauf derartiger

kennlinien S-förmig, wobei der zentrale Bereich dieser S-Kurve mehr oder weniger weit asymptotisch einer Geraden angepasst ist, die als Tangente an den Wendepunkt bekannt ist. Die Steigung dieser Tangente ist ein bewährter Index zur Beurteilung der Leistungsdynamik des geprüften Systems. Ein besonderer Vorteil dieser Betrachtungsweise beruht darauf, dass bei der statistischen Untersuchung einer Häufigkeitsverteilung in Form der Kennlinie vom Zentrum weiter entfernte, oft schwerer messbare Randwerte vernachlässigt werden können. Gerade diese Werte können die genaue Festlegung der bisher gebräuchlichen Nystagmusedauer behindern, die ohnehin am aussageschwächsten unter den Nystagmusparametern Dauer, Frequenz und Amplitude steht (Henriksson *et al.* 1967, Mittermaier 1965, Torok, 1957). Als ein maximales Erregungsmuster des geprüften Vestibulärorgans könnte man die Kulmination der postkalorischen Schlagrate bezeichnen. Diese Tatsache wurde auch von Hinchcliffe (1967) besonders herausgestellt. Wie er nachweisen konnte, besteht eine hohe Korrelation zwischen der Geschwindigkeit der langsamen Phase im Elektronystagmogramm und der Frequenz in einem 30-Sekunden Intervall um den Kulminationspunkt herum. Die Frequenzverteilung soll in diesem Bereich einer Gaußschen Verteilung entsprechen.

Besonders günstig für den Beobachter verhält es sich, dass die maximale Nystagmusfrequenz nur sehr selten fünf Schläge/sec erreicht oder sogar höher liegt. Fünf Schläge pro sec kann der geübte Beobachter aber z. B. mit der Eichtafel eines kleinen EKG-Gerätes einer Zeitkurve aufdrucken oder nach Zeitklassen geordnet mitzählen.

Begrenzt man den zentralen Bereich der oben genannten S-förmigen Kennlinie zeitlich im Bereich des angenähert geradlinigen Verlaufes, so entspricht die Steigung dieser angenäherten Geraden dem Tangens des Quotienten von abgegrenzten Einzelschlägen zu gleichermassen abgegrenzten Sekunden. Wählt man entsprechend den statistischen Versuchserfahrungen ein stets gleichgroßes Zeitintervall zum Zählen der Nystagmusschläge während der Frequenzkumulation, dann lässt sich diese Schlagrate so in einem rechtwinkligen Koordinatenschema abbilden, dass die Kennlinienfunktion als Zeiger zur Darstellung kommt (Abb. 1).

Empirisch konnte entsprechend den Ergebnissen von 136 postkalorischen Frequenzzeitkurven die vom Verfasser im klinischen Routinebetrieb mit Frenzel-Brille und Eichtafel eines Zeitschreibers ermittelt wurden, der Zeitraum der bei der Zeigerdarstellung verwendeten Frequenz auf 30 sec begrenzt werden. Im Zentrum dieser 30-Sekunden Strecke befinden sich die leicht auffindbaren 10 Sekunden höchster postkalorischer Nystagmusfrequenz, hinzugefügt wurden die beidseits benachbarten 10-Sekunden, um dem erhaltenen Wert eine breitere Basis zu geben. Zur graphischen Ermittlung der Kennlinie werden auf der Abszisse eines rechtwinkligen Koordinatensystems die Einzelsekunde und auf der Ordinate der Einzelschlag als gleichgroße Einheiten gewählt. Da nun bei allen Versuchen ein immer gleicher Zeitraum zugrunde gelegt wird, nämlich die zentralen 30 Sekunden,



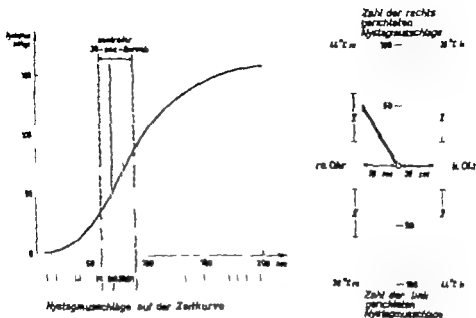


Abb. 1

den, kann man stets eine Ordinaten-Parallele durch den 30-Sekundenpunkt der Abszisse benutzen, um auf ihr die gefundene Schlagzahl während des zentralen 30-Sekundenbereiches aufzutragen. Verbindet man den Schlagzahlpunkt mit dem Nullpunkt des Koordinatensystemes, so hat man die Kennlinie für das postkalorische Frequenzverhalten annähernd festgelegt (Abb. 1).

Die vier Kennlinienaeizer, die man ermittelt, nachdem nacheinander beidseits 30 sec lang mit  $30 \pm 20$  ml  $44^\circ\text{C}$  und  $30^\circ\text{C}$  warmen Wassers gespült wurde, lassen sich so auf die vier Quadranten eines rechtwinkligen Koordinatensystems erteilen, dass links von der Ordinaten die Ergebnisse der kalorischen Prüfung des rechten Ohres, rechts von der Ordinaten die Ergebnisse der kalorischen Prüfung des linken Ohres, oberhalb der Abszisse die Ergebnisse bei rechtsgerichtetem Nystagmus und unterhalb der Abszisse die Ergebnisse bei linksgerichtetem Nystagmus angeordnet werden, d. h. im I Quadranten wird die Kennlinie nach Kaltspülung links, im II Quadranten die Kennlinie nach Warmspülung rechts, im III Quadranten die Kennlinie nach Kaltspülung rechts und im IV Quadranten die Kennlinie nach Warmspülung links ermittelt (Abb. 1).

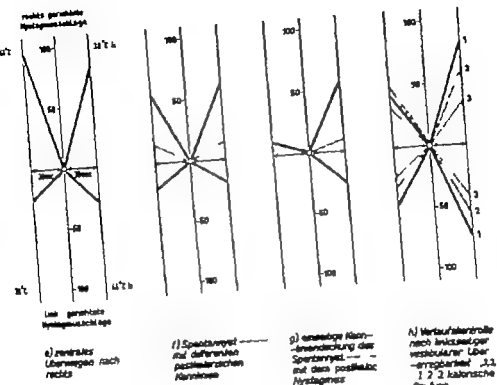
Markiert man sich an der schlagratenzählenden Ordinatenparallele noch die mittlere Schlagrate  $\pm$  der Standardabweichung als Maß der Streuung, so betrug bei unseren 136 kalorischen Prüfungen die mittlere Schlagrate der zentralen 30 Sekunden  $40 \pm 20$  Schläge — so kann auch der weniger Gebildete das komplette Ergebnis der 4 zusammenhängenden kalorischen Vestibularprüfungen beurteilen.

kennlinien S-förmig, wobei der zentrale Bereich dieser S-Kurve mehr oder weniger weit asymptotisch einer Geraden angepasst ist die als Tangente an den Wendepunkt bekannt ist. Die Steigung dieser Tangente ist ein bewährter Index zur Beurteilung der Leistungsdynamik des geprüften Systems. Ein besonderer Vorteil dieser Betrachtungsweise beruht darauf dass bei der statistischen Untersuchung einer Häufigkeitsverteilung in Form der Kennlinie vom Zentrum weiter entfernte, oft schwerer messbare Randwerte vernachlässigt werden können. Gerade diese Werte können die genaue Festlegung der bisher gebräuchlichen Nystagmusedauer behindern, die ohnehin am aussageschwächsten unter den Nystagmusparametern Dauer, Frequenz und Amplitude steht (Henriksson *et al.*, 1967; Mittermaier 1965; Torok, 1957). Als ein maximales Erregungsmuster des geprüften Vestibularorgans konnte man die Kulmination der postkalorischen Schlagrate bezeichnen. Diese Tatsache wurde auch von Hinchcliffe (1967) besonders herausgestellt. Wir er nachweisen konnte besteht eine hohe Korrelation zwischen der Geschwindigkeit der langsamen Phase im Elektronystagmogramm und der Frequenz in einem 30-Sekunden Intervall um den Kulminationspunkt herum. Die Frequenzverteilung soll in diesem Bereich einer Gaußschen Verteilung entsprechen.

Besonders günstig für den Beobachter verhält es sich, dass die maximale Nystagmusfrequenz nur sehr selten fünf Schläge/sec erreicht oder sogar höher liegt. Fünf Schläge pro sec kann der geübte Beobachter aber z.B. mit der Eichtafel eines kleinen EKG-Gerätes einer Zeitkurve aufdrucken oder nach Zeitklassen geordnet mitzählen.

Begrenzt man den zentralen Bereich der oben genannten S-förmigen Kennlinie zeitlich im Bereich des angenähert geradlinigen Verlaufes, so entspricht die Steigung dieser angenäherten Geraden dem Tangens des Quotienten von abgegrenzten Einzelschlägen zu gleichermassen abgegrenzten Sekunden. Wählt man entsprechend den statistischen Versuchserfahrungen ein stets gleichgroßes Zeitintervall zum Zählen der Nystagmusschläge während der Frequenzkumulation, dann lässt sich diese Schlagrate so in einem rechtwinkligen Koordinatenschema abbilden, dass die Kennlinienfunktion als „Zeiger“ zur Darstellung kommt (Abb. 1).

Empirisch konnte entsprechend den Ergebnissen von 136 postkalorischen Frequenzzeitkurven, die vom Verfasser im klinischen Routinebetrieb mit Frenzel Brille und Eichtafel eines Zeitschreibers ermittelt wurden, der Zeitraum der bei der Zeigerdarstellung verwendeten Frequenz auf 30 sec begrenzt werden. Im Zentrum dieser 30-Sekunden Strecke befinden sich die leicht auffindbaren 10-Sekunden höchster postkalorischer Nystagmusfrequenz, hinzugefügt wurden die beidseits benachbarten 10 Sekunden, um dem erhaltenen Wert eine breitere Basis zu geben. Zur graphischen Ermittlung der Kennlinie werden auf der Abszisse eines rechtwinkligen Koordinatensystems die Einzelsekunde und auf der Ordinate der Einzelschlag als gleichgroße Einheiten gewählt. Da nun bei allen Versuchen ein immer gleicher Zeitraum zugrunde gelegt wird, nämlich die zentralen 30 Sekunden



$\bar{x} \pm s$

Abb. 3.

#### 4 Nistisches Richtungsüberwiegen.

- (a) Verstärkung einer Nystagmusrichtung — die Maximumschlagraten der Warmprüfung eines Ohres und der Kaltprüfung des kontralateralen Ohres überschreiten den Normbereich (Abb 3 e)
- (b) Unterdrückung einer Nystagmusrichtung — die Maximumschlagraten der Warmprüfung eines Ohres und der Kaltprüfung des kontralateralen Ohres unterschreiten den Normbereich.
- 3 Überlagerung durch einen Spontannystagmus. Die Frequenz des Spontannystagmus wird vor der kalorischen Prüfung über 30 sec ausgemittelt. Die beiden erhaltenen Schlagraten werden in demselben Schema in zwei Quadranten abgetragen, so dass zwei richtungsentsprechende Kennlinien entstehen. Anschließend werden in demselben Schema die Ergebnisse der kalorischen Prüfung eingetragen. Beim Vergleich der postkalorischen und des Spontannystagmus ergeben sich folgende Möglichkeiten
- (a) Kennliniendifferenz mit Verstärkung des dem Spontannystagmus gleichgerichteten postkalorischen Nystagmus (Abb. 3 f)

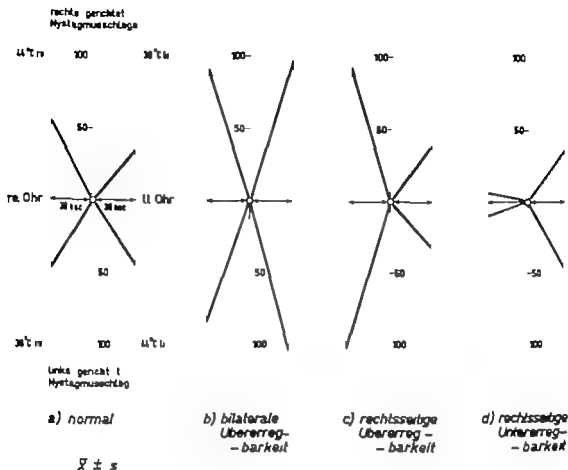


Abb. 2.

Teils als Ergebnis unserer Untersuchungen und teils aufgrund theoretischer Überlegungen entstehen bei Anwendung der oben beschriebenen Methode typische optisch einprägsame Muster die entweder rein oder als Mischformen auftreten können. Diese Muster sollen im folgenden schematisch zusammengestellt werden

- 1 Normale kalorische Erregbarkeit beider Vestibularorgane. Die Maximumschlagraten befinden sich für alle vier Prüfungen im Normbereich (Abb 2 a)
- 2 Bilaterale Erregbarkeitsveränderungen
  - (a) bilaterale Übererregbarkeit — die Maximumschlagrate überschreitet allseitig den Normbereich (Abb 2 b)
  - (b) bilaterale Untererregbarkeit — die Maximumschlagrate unterschreitet allseitig den Normbereich
- 3 Periphere Seitendifferenzen
  - (a) einseitige Übererregbarkeit — die Maximumschlagraten der Warm und kaltprüfung eines Ohres überschreiten den Normbereich (Abb 2 c)
  - (b) einseitige Untererregbarkeit — die Maximumschlagraten der Warm und kaltprüfung eines Ohres unterschreiten den Normbereich (Abb 2 d)

LITERATUR

- BARRE, J. H., and CHAMPTON, G. H. 1944 Quantification of the human nystagmic response to angular acceleration. Prediction formulas and nomograph. *Acta Otolaryng* (Stockh.) 52, 335.
- CAW, E. L., DAWE, F. A., and MARFIELD, M. W. 1940: *Statistics manual*. Dover Publications, Inc., New York.
- DECKER, H. 1945 *Vestibularis-Funktionstest mittels Nystagmographie*. Dr. A. Hähig Verlag Heidelberg.
- FITZGERALD, G. and HALLPIPER, C. S. 1942 Studies in human calicular function. I. Observations on the directional preponderance of caloric nystagmus resulting from cerebral lesions. *Brain*, 65, 116.
- HERNIMON, V. G., KOSOV, R., and FRANKLNER, C. 1961 Studies on habituation of vestibular reflexes. I. Effect of repetitive caloric test. *Acta Otolaryng* (Stockh.) 52, 332.
- HERNIMON, V. G., LINDHART, S., LÖNNBERG, K., and JONSSON, A. 1967 New techniques of otoneurological diagnosis. I. Analysis of eye movements. *Myogenic, kinesthetic and vestibular mechanism* (Ciba Foundation Symposium) 5, 319. Churchill, London.
- HERNIMON, V. G., JONSSON, A., and ÖRTENGREN, H. 1967 New techniques of otoneurological diagnosis. II. Vestibulo-spinal and postural patterns. *Myogenic, kinesthetic and vestibular mechanism* (Ciba Foundation Symposium) 5, 331. Churchill, London.
- MURCHLOVE, R. 1947 Validity of measures of caloric test response. *Acta Otolaryng* (Stockh.) 53, 89.
- 1948 Nystagmus test as an index of caloric test response. *Acta Otolaryng* (Stockh.) 53, 311.
- JIYUO, K. 1967 Methodik und Bewertung der Vestibularisdiagnostik. *Med. Na Medica Vardmark*, 19, 463.
- KONRADT, H. H. 1946 Physiologie und Klinik des zentral-otolithären Systems (Blick auf Stützmotoren). Handbuchbeitrag aus Hals-Nasen-Ohrenheilkunde. *Karger'sches Handbuch*, 3, 2, 2150. Thieme-Verl., Stuttgart.
- MITTELMANN, R. 1945 Der experimentellen III. Sehversuchsprüfung. Handbuchbeitrag aus Hals-Nasen-Ohrenheilkunde. *Karger'sches Handbuch*, 3, 1, 381. Thieme-Verl., Stuttgart.
- MITTELMANN, R., and CHRISTIAN, W. 1951 Dauer Schlagzahl und Gesamtempfindung des kalorischen Nystagmus. *Z. Laryng. Rhinol.*, 71, 30.
- PRALTE, C. R. and OLSEN, R. 1962: D. pathologische kalorische Labyrinthreaktion. *Arch. Ohr. Nas. K. H. H. H. H.* 170, 325.
- PROBEN, P. *Regelungs- und Steuerungstechnik*. Aus Fischer Lexikon Technik 2. Fischer Buchverl. K. G. Frankfurt und Hamburg.
- STALK, J. 1954 Electro-nystagmography in the caloric and rotatory test. *Acta Otolaryng* (Stockh.) 5, ppl. 137 &.
- TOMON, K. 1957 The culmination phenomenon and frequency pattern of the thermal nystagmus. *Acta Otolaryng* (Stockh.) 48, 330.
- TOMON, K. and ASADA, P. 1961 Frequency test of Nystagmus Analysis. *Acta Otolaryng* (Stockh.) 51, 343.
- TRIMMER, D. 1945 Physiologie des Gleichgewichtsorganen. Handbuchbeitrag aus Hals-Nasen-Ohrenheilkunde. *Karger'sches Handbuch*, 3, 1, 311. Thieme-Verl., Stuttgart.

D. Dr. C. Claassen,  
Ruhensstr. 63  
1 Berlin 41, Deutschland

Erstveröffentlichung am 28. September 1968

- (b) kennliniendifferenz mit Unterdrückung des dem Spontannystagmus entgegengerichteten postkalorischen Nystagmus (Abb 3 f)
  - (c) einseitige kennliniendeckung des Spontannystagmus und des gleichgerichteten postkalorischen Nystagmus einer Seite ohne Richtungsumkehr des Spontannystagmus bei entgegengesetzter kalorischer Prüfung auf der gleichen Seite (Abb 3 g) (Meist treten (a) und (b) vergesellschaftet auf)
- 6 Vergleich mehrerer Untersuchungen an demselben Patienten
- (a) Verlaufskontrolle mittels der kennlinienzeigerbewegung bei Ausbildung oder Rückbildung einer der obengenannten Störungen (Abb 3 h)
  - (b) Adaptationstest nach Henriksson *et al* (1961) kennlinienvergleich der 1., 2., 3., und der *n*ten kalorischen Prüfung desselben Patienten am gleichen Tage.

Aussagen über den Erfolg kalorischer Reizung bei Vorliegen eines Spontannystagmus lassen sich nach der üblichen Hallpike-Methode nur treffen, wenn es zu einer Nystagmusumkehr kommt während mit dieser Methode quantitative Unterschiede zwischen dem gleichgerichteten spontanen und postkalorischen Nystagmus herausgearbeitet werden können.

Technisch bessere Ergebnisse als das reine Abschätzen des Maximumbereiches und sein Auszählen nach der Stoppuhr liefert das Aufdrucken der einzelnen beobachteten Nystagmusschläge auf eine Zeitkurve, die anschließend ausgezählt wird. Bei unseren Versuchen lag das mittlere Maximum der Schlägenfrequenz des postkalorischen Nystagmus  $1 \pm$  Standardabweichung  $77 \pm 18$  sec nach Spülbeginn. Bei Auszählung des Frequenzmaximums nach der Stoppuhr erscheint uns der Bereich zwischen 60–90 Sekunden nach Spülbeginn wichtig.

Möglicherweise kann diese Methode auch zur Beurteilung der Geschwindigkeit der mit dem Elektronystagnogramm oder Photoelektronystagnogramm registrierten langsamen und schnellen Phase des postkalorischen Nystagmus I benutzt werden.

## SUMMARY

Using a strip-chart recorder and FRENZEL glasses, the frequency of post caloric nystagmus is manually plotted on the constantly moving chart. From a sample of 136 cumulative frequency distributions have been constructed according to each frequency strip. The important central parts of the S-shaped cumulative distributions approximate linear curves, especially during the central 30 seconds. As a practical application the tangent from the beats on the central 30 seconds easily can be sketched in a rectangular coordinate system. This is then used as an index of one caloric test. If arranging the indexes from the four tests of one patient on the four quadrants of the coordinate system the result of the whole calorisation is transformed into a graphical pattern resembling a butterfly. Different shapes are associated with different diseases.

# LITERATUR

- BROWN, J. H., und CRAWFORD, G. H. 1964 Quantification of the human ystagic response to angular acceleration. Prediction formulae and nomograph. *Acta Otolaryng* (Stockh.) 58 553.
- CROW, E. L., DICK, P. A., und MAXFIELD, M. W. 1960 *Statistics manual*. Dover Publications, Inc., New York.
- DECKERT, H. 1965 *Vestibular-Funktionstestung mittels Nystagmographie*. Dr. A. Hähig-Verl. Heidelberg.
- FITZGERALD, G. und HANAUER, C. S. 1942 Studies I human vestibular function. I. Observations on the directional preponderance of caloric nystagmus resulting from cerebral lesions. *Brain*, 65, 178.
- HANAUER, C. S., ROYCE, R., und FERNANDEZ, C. 1961 Studies habituation of vestibular reflexes. I. Effect of repetitive caloric test. *Acta Otolaryng* (Stockh.) 53, 333.
- HANAUER, C. S., LUNDGREN, S., LUNDGREN, K., und NILSSON, A. 1967 New techniques of otoneurological diagnosis. I. Analysis of y movements. *Myologic, kinesthetic and vestibular mechanisms* (Giba Foundation symposium) 8, 219 Churchill, London.
- HANAUER, C. S., LUNDGREN, G., und GÖRANSSON, R. 1967 New techniques I otoneurological diagnosis. II. Vestibulo-spinal and postural patterns. *Myologic, kinesthetic and vestibular mechanisms* (Giba Foundation symposium) 8, 231 Churchill, London.
- HINCHCLIFF, R. 1967 Validity of measures of caloric test response. *Acta Otolaryng* (Stockh.) 63, 66.
- 1968 Nystagmus rate index of caloric test response. *Acta Otolaryng*. (Stockh.) 63, 311.
- JÄGER, K. 1967 Methodik und Bewertung der Vestibulardiagnostik. *Medizinische Fortschritt* 19 443.
- KÖRNER, H. H. 1964 Physiologie und Klinik des neutralen vestibulären Systems (Büchlein und Stützmotoren) Handbuchbeitrag aus Hals-Nasen-Ohrenheilkunde Kurzgefaßtes Handbuch, 3, 2154. Thieme-Verl., Stuttgart.
- MITTELMANN, R. 1965 Die experimentellen Gleichgewichtsprüfungen. Handbuchbeitrag aus Hals-Nasen-Ohrenheilkunde. Kurzgefaßtes Handbuch, 3, 1 381 Thieme-Verl., Stuttgart.
- MITTELMANN, R., und CHRISTIAN, W. 1934 Dauer Schlingentest und Gesamtamplitude des kalorischen Nystagmus. *Z. Laryng. Rhinol.* 23, 26.
- PRAL, C. R., und GÖRANSSON, R. 1962 Die pathologische kalorische Labyrinthreaktion. *Arch. Otorhinolaryng.* 179 523.
- PROUSE, P. *Regulation and Stimulation of the Vestibular System*. 2, Fischer Buchverl. K. G., Frankfurt und Hamburg.
- SCHULZ, J. 1956 Electronystagmography in the caloric and rotatory test. *Acta Otolaryng* (Stockh.) Suppl. 137 8.
- TOMON, M. 1937 The culmination phenomenon and frequency pattern of the thermic nystagmus. *Acta Otolaryng* (Stockh.) 42, 339.
- TOMON, M., und NYKEL, P. 1961 Frequency meter for Nystagmus Analysis. *Acta Otolaryng* (Stockh.) 56 363.
- TRINCHARD, D. 1966 Physiologie des Gleichgewichtsorgans. Handbuchbeitrag aus Hals-Nasen-Ohrenheilkunde Kurzgefaßtes Handbuch, 3, 1, 211 Thieme-Verl., Stuttgart.

D. M. d. C. Clausen,  
Humboldt 64,  
1 Berlin 41 Deutschland

Eingegangen am 20. September 1968

## COMBINED ACTION OF LARYNGEAL MUSCLES

H. R. KONRAD and C. C. RATTENBORG

*From the Sections of Otolaryngology and Anesthesiology University of Chicago  
Chicago Ill., U.S.A*

In the lightly halothane anesthetized dog the cricothyroid and posterior crico-arytenoid muscles are active simultaneously. With electrical stimulation of the cricothyroids, laryngeal resistance is increased and cross sectional area of the larynx decreased. With electrical stimulation of the posterior crico-arytenoids, laryngeal resistance is decreased and cross sectional area of the larynx increased. Stimulation of the cricothyroid and posterior crico-arytenoid muscles simultaneously results in an even lower airway resistance and an even larger cross sectional area than the posterior crico-arytenoid muscles can accomplish alone. This synergism is described and illustrated.

The respiratory function of the larynx is that of a variable resistance in the upper airway. The changes in resistance are carried out by the coordinated activity of intrinsic and extrinsic muscles of the larynx. Through studies of anatomy, nerve cutting, nerve and muscle stimulation and electromyography the individual activity of the laryngeal muscles has been described (Nakamura *et al* 1938, Negus, 1962, Brewer *et al* 1962, 1963, Rattenborg *et al* 1963). However laryngeal muscles do not act in an isolated manner. In order to understand laryngeal dynamics we must determine how laryngeal muscles act in combination.

From electromyographic studies in the dog, under light halothane anesthesia, we found that the cricothyroid and the posterior crico-arytenoid muscles usually act simultaneously during inspiration. The paradox of a closing muscle (cricothyroid) acting simultaneously with an opening muscle (posterior crico-arytenoid) during inspiration (when the larynx opens) suggested further studies (Fig. 1).

### METHODS

#### *In situ larynx*

Four dogs under halothane anesthesia with denervated larynx had electrodes inserted in both cricothyroid and both posterior crico-arytenoid muscles for stimulation. Laryngeal resistance was measured as the pressure drop across the larynx through which a constant airflow was passed.

This work was in part supported by LSPHS NB03121-12 and LSPHS 5R01HE0037-6-03.



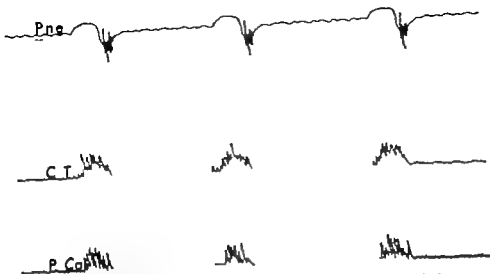


FIG. 1. The pneumotachograph runs with inspiration up CT integrated electromyograph of the cricothyroid muscles; PCA; integrated electromyograph of the posterior cricoarytenoid muscle

### Isolated larynx

The larynx was removed from two anesthetized dogs rapidly electrodes inserted as above and movies taken during stimulation

In both experiments a Dism Multistim was used. A diphasic impulse at 100 cps and 0.2 msec duration was used. Voltage could be varied individually to each stimulating electrode through a rheostat from 0-10 vts.

### PROCEDURE

The cricothyroid and posterior cricoarytenoid muscles were stimulated first individually and later simultaneously

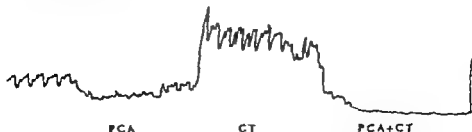


FIG. 2. Traces of laryngeal resistance during muscle stimulation measured as pressure drop across the larynx during constant flow PCA stimulation of posterior cricoarytenoid CT stimulation of cricothyroid PCA and CT simultaneous stimulation of posterior cricoarytenoid and cricothyroid.

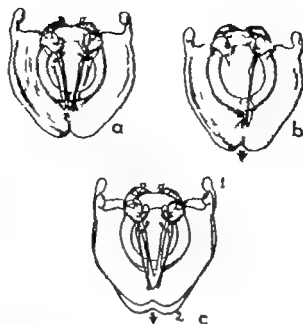


FIG. 3 (a) Opening of larynx by posterior crico-arytenoid (b) cricothyroid increases AP diameter but glottis partially closed (c) synergistic action of both muscles results in greatest cross sectional area.

## RESULTS

Stimulating the cricothyroid muscles individually increased the laryngeal resistance in the *in situ* preparation. In the isolated larynx preparation the anterior/posterior diameter increased but vocal cords were approximated causing a decreased cross sectional area.

Stimulating the posterior crico-arytenoids alone reduced laryngeal resistance in the *in situ* preparation and opened the vocal cords in the isolated larynx.

Balanced stimulation of cricothyroids and posterior crico-arytenoids together resulted in the lowest resistance recorded *in situ* and in the greatest cross sectional area in the isolated larynx (Figs. 2 and 3)

## DISCUSSION

The apparent paradox of a closing muscle (cricothyroid) and an opening muscle (posterior crico-arytenoid) acting simultaneously during inspiration is resolved by the finding that they can act synergistically to provide greater opening of the larynx than the posterior crico-arytenoids can alone.

## ZUSAMMENFASSUNG

Die Kehlkopfmuskeln Krikothyroideus und Krikoarytenoideus Posterior sind im Hunde unter Halothane Narkose zur selben Zeit aktiv. Während elektrischer Reizung des Krikothyroideus geht der Luftwiderstand des Kehlkopfes hoch und

die Fläche der Öffnung nimmt ab. Während elektrischer Reizung des Krikoarytenoideus Posterior geht der Luftwiderstand des Kehlkopfes ab und die Fläche der Öffnung wird grösser. Wenn Krikothyroideus und Krikoarytenoideus Posterior zur selben gereizt werden, wird der Luftwiderstand am geringsten, und die Fläche der Öffnung die grösste. Die Synergie dieser Muskeln ist beschrieben und illustriert.

## REFERENCES

- BERTER, D. W., FRANKS-ANDERSEN, K., and DANA, S. T. 1962: Investigations I laryngeal physiology: The canine larynx. I. *Trans. Am. Laryng. Ass.*, 53: 174.  
 — 1963: Investigations in laryngeal physiology: The canine larynx. II. *Ann. Otol.* 72, 1960.  
 ARAKAWA, F., UEDA, Y. and SONODA, Y. 1956: Electromyographic study on respiratory movements of the intrinsic laryngeal muscles. *Laryngoscopy* 66: 109.  
 KELLER, M. E., 1962: *Comparative anatomy and physiology of the larynx*. Hafner Publishing Co., New York.  
 RATTENBORN, C. C., BARTON, M. D., KALK, M. L., LOGAN, W. J., KOSMAN, H. R., and HOLADA, D. A., 1962: Reflex activity of the larynx during breathing. *Anesthesiology* 24: 1.  
 RATTENBORN, C. C., BARTON, M. D., KOSMAN, H. R., KALK, M. L., and HOLADA, D. A., 1963: Laryngeal activity during normal and labored breathing in anesthetized dogs. *Fed. Proc.* 22, 2 (Abstract).

H. R. Kosman, M.D.  
 Section of Otolaryngology  
 University of Chicago,  
 840 East 58th Street  
 Chicago III., 60637 U.S.A.

Received August 9 1968

## SALIVARY-GLAND SCANNING WITH $^{99m}\text{Tc}$

B ENFORS, M LIND and B SÖDERBORG

*From the Departments of Otolaryngology and Radiology Södersjukhuset  
Stockholm, Sweden*

$^{99m}\text{Tc}$  pertechnetate concentrates in the salivary glands and offers a possibility of scanning the glands. The difficulty of oral cavity contamination is overcome by giving 1.0 mg atropine intravenously before  $^{99m}\text{Tc}$  administration.

Many salivary gland diseases are associated with functional disturbances. Their demonstration can often make a valuable contribution to the sometimes difficult diagnosis (Diamant, 1960). Such disturbances are, however, difficult to establish clinically. Deviations from the normal must be considerable if they are to be demonstrated by means of the quantitative and qualitative studies of the saliva hitherto used (Seige & Pfeiffer 1955; Seige, 1956; Enfors, 1962). The examinations are generally time-consuming, since the recordings must be made both at rest and under various degrees and types of salivary gland stimulation. Moreover, the saliva from the individual glands must be collected separately. Consequently, the need exists for a new functional test.

Since the radioactive isotope  $^{99m}\text{Tc}$  (technetium  $^{99m}\text{Tc}$ )—which was introduced in the early 1960s—is, in its pertechnetate form, concentrated in the salivary glands among other sites, it permits salivary gland scanning (Harper *et al.*, 1962). Technetium is an element in group VII and is formed when  $^{99}\text{Mo}$  molybdenum decomposes into  $^{99m}\text{Tc}$  technetium which, in turn, decomposes into  $^{99}\text{Tc}$  technetium. The half life of  $^{99m}\text{Tc}$  is short, i.e., only 6 hours. The isotope emits a  $\gamma$ -radiation of 140 keV of which 8–9% is converted into  $\beta$ -radiation (Harper *et al.* 1965; Smith, 1964).

In view of the short half life, the total body irradiation is low, even if a relatively large quantity of  $^{99m}\text{Tc}$  is injected. One millicurie gives a total body irradiation of about 20 millirad. The thyroid gland and stomach, whose irradiation is greatest, receive from 200–300 millirad. As a comparison, it can be mentioned that a thyroid scan with 50 microcurie of  $^{131}\text{I}$  gives a total thyroid irradiation which is approximately 1000 times greater and that roentgenological examination of the stomach gives a dose over this organ that is about 10–20 times greater (Harper *et al.*, 1965).

Pertechnetate  $^{99m}\text{Tc}$  is distributed in the body in almost exactly the same way as  $^{131}\text{I}$ . Thus, the isotope is concentrated in the gastric mucosa, and in the thyroid and salivary glands (Harper *et al.*, 1965; Andros *et al.*, 1965).

hazem *et al* 1967) Although occasional salivary gland scans have been published earlier the records have differed in appearance, due partly to the fact that  $^{99m}\text{Tc}$  had collected in the oral cavity to a varying high degree, one of the results being that the concentration in the salivary glands varied appreciably (Börner *et al.*, 1965 Harden *et al* 1967) Harden stated that, on one occasion, premedication with 0.5 mg of atropine led to the amount of  $^{99m}\text{Tc}$  decreasing in the oral cavity We have tried to devise a method in which salivary-gland scanning can be performed routinely with consistently good records, without contamination of the oral cavity by  $^{99m}\text{Tc}$ .

### MATERIAL AND METHODS

Per technetate  $^{99m}\text{Tc}$  was obtained from  $^{99m}\text{molybdenum}$  piles delivered by AB Atomenergi, Studsvik, Sweden. The isotope was prepared daily and sterilized with the help of a Millipore® filter The procedure takes about 15 minutes. Totally 60 subjects were examined. Only a few of them had symptoms of salivary-gland disease.

In most cases, the examination was started with slow intravenous injection of 10 mg of atropine sulphate. After about 1 min,  $^{99m}\text{Tc}$  was injected intravenously through the same cannula. The dose of  $^{99m}\text{Tc}$  ranged from 1 to 6 millicurie generally 2.0 millicurie. The scanning, which was started 15 minutes after injection of  $^{99m}\text{Tc}$ , occupied approximately 20 minutes. It was performed with a Lucab whole-body scanner provided with a 3-inch Na crystal and single-channel analyzer and an 11-hole collimator (rate 4 mm/second, distance between the lines 3 mm) In most cases, two or more scans were made in direct succession Since many of the subjects had difficulty in keeping their head still during the recording, the head was fixed in a stand constructed for the purpose

### RESULTS

At the first examinations, made without preceding atropinization, good pictures of the salivary glands were sometimes obtained, but the scan had a varying appearance and was not easily reproducible. Records made in direct succession in the same subject showed that they rapidly changed in appearance and that considerable increasing activity occurred in the oral cavity (Fig 1) Since this increasing activity could be presumed to depend on transport of  $^{99m}\text{Tc}$  with the saliva from the salivary glands to the oral cavity atropine was given to inhibit the salivary flow. A small dose of atropine (0.5 mg intramuscularly) only occasionally prevented contamination of the oral cavity and the records continued to vary in appearance (Fig 2) When the dose was raised to 1.0 mg of atropine sulphate intramuscularly it was exceptional for the  $^{99m}\text{Tc}$  to leak into the oral cavity despite the higher dose. The salivary glands appeared consistently and the records



FIG. 1. Without atropinization, contamination of the oral cavity often makes the record unusable.

FIG. 2. With unsatisfactory tropicization, contamination of the oral cavity occasionally occurs. The record is taken immediately after successful tropicization (the first of the two). In this case, mainly the submandibular glands contribute to the oral contamination.

FIGS. 3 and 4. With 10 mg of tropicization intra-nasally, contamination of the oral cavity was regularly inhibited.

were of good quality (Figs. 3 and 4). The rare leakage into the oral cavity resulted in a decrease in the  $^{99m}\text{Tc}$  content of the salivary glands (Fig. 2).

In the subjects without symptoms of salivary-gland disorders, the uptake of  $^{99m}\text{Tc}$  was essentially the same bilaterally. Variations were, however, present between the uptake in the parotid and the submandibular glands (Figs. 3 and 4). The uptake also varied between different subjects. A possible explanation of a low uptake in the salivary glands, without contamination of the oral cavity was that the atropine inhibited the glandular activity so greatly in certain individuals that the uptake of  $^{99m}\text{Tc}$  was also affected. To rule out this possibility a new recording was made in such cases without atropinization after a day or so had elapsed. In no case did we find that the low uptake could be ascribed to excessive atropine medication. Furthermore a higher dose than 10 mg of atropine sulphate intravenously was never given.

### DISCUSSION

The scanning method described can be used routinely to visualize the salivary glands. The standard dose—2.0 millicurie of  $^{99m}\text{Tc}$ —which gives low total body irradiation, has proved sufficient to give good, uniform recordings. If greater resolution is desired to permit a more detailed analysis, the dose can be raised to 8 millicurie and the rate of scanning be decreased. The degree of density of the records is dependent partly on the total quantity of  $^{99m}\text{Tc}$  injected, and partly on the relative concentration of the isotope in the parenchyma of the salivary glands. The uptake in these glands can be presumed to vary with their different functional and pathological state. In occasional examination of patients with symptoms of salivary-gland disease, records differing from those in healthy subjects were obtained. It is possible that this scanning method may assume importance for the evaluation of such pathological conditions.

### ZUSAMMENFASSUNG

$^{99m}\text{Tc}$  per technetate wird in den Speicheldrüsen akkumuliert und bietet eine Möglichkeit diese Drüsen szintigraphisch zu untersuchen. Die Schwierigkeit der Mundkavität zu überwinden wird überwunden, indem man 1,0 mg Atropin intravenös, oder der Verabreichung von  $^{99m}\text{Tc}$  gibt.

### REFERENCES

1. DODD, H., HANSEN, P. V., LATIMER, L. A., and MCCABER, R. J. 1965 Per technetate-99m for visualization of man with applications to thyroid scanning and the study of thyroid physiology. *J. Clin. Endocrin.*, **25**, 1067.
2. LA, W. G., MANN, H., and MOEL, E. 1965 Die szintigraphische Darstellung der Kopf speicheldrüsen mit Technetium 99m. *Med. W. H.*, **42**, 2378.
3. DIAM, H. 1964: Enlargement of the parotid gland. *Acta Otolaryng. (Stockh.)*, **53**, 220.
4. FURUKAWA, H. 1963 The parotid and submandibular secretions in man. *J. Otolaryng. (Stockh. Suppl.)*, **152**.

- HARDEN, R. McG. HILDITCH, T. E., KENNEDY, I., MASON, D. K., PAPADOPOULIS, S., and ALEXANDER, W. D. 1967. Uptake and scanning of the salivary glands in man using per technetate  $^{99m}\text{Tc}$ . *Clin. Sci.*, 32, 49.
- HARPER, P. V., ANDROS, G., LATHEOF, K. A., SIEMENS, W., and WEISS, L., 1962. Metabolism of technetium-99. (Abstr.) *Radiat. Res.*, 16, 292.
- HARPER, P. V., LATHEOF, K. A., JIMENEZ, F., FINE, R., and GOTTSCHALK, A., 1963. Technetium 99m as a scanning agent. *Radiology* 83, 101.
- KAZEM, I., GELINANT, P., and SCHENK, P., 196. Organ visualization with technetium 99m preparations. *Brit. J. Radiol.*, 40, 292.
- SEIGE, K., 1956. Zur Physiologie und Pathologie der Parotissekretion. *Verhandlungen der Deutschen Gesellschaft für innere Medizin*, 62. Kongress.
- SEIGE, K., and PRÄTZER, K., 1955. Untersuchungen über die Funktion der Ohrspeicheldrüse. Pilocarpintest und Sialographie bei Erkrankungen der Parotis und Parotidbereich. *Dtsch. Z. Verdau. Stoffwechselkr.*, 15, 86.
- SMITH, E. M. 1964. Properties, uses, radiochemical purity and calibration of  $\text{Tc}^{99m}$ . *J. Nucl. Med.* 5, 871.

B. Enfors, M.D., Dept. of Otolaryngology  
Södersjukhuset Stockholm, Sweden

Received August 26, 1968



# CALCIOPEN®-K

## tabletter à 600 000 IE

Jämförande antibakteriella aktivitetsbestämningar har bekräftat de kliniska iakttagelserna att V penicillin fortfarande försvaret sin plats som förstahandspreparat vid penicillinbehandling. För att säkerställa terapi svar även vid infektioner förorsakade av stammar med något mindre känslighet för penicillin har man mer och mer övergått till högre dosering.

**CALCIOPEN® K** tablett innehåller 600 000 IE. Den är spolformad filmdragerad och lätt att svälja.

### DEKLARATION

Varje tablett innehåller  
Phenoxymethylpenicillinkali m 0,4 g  
(responder 600 000 IE)

### INDIKATIONER

All infektionssjukdom förorsakad av penicillinkänsliga bakterier

### KONTRAINDIKATION

Penicillinöverkänslighet

### DOSERING

Vuxna: 4 tabletter per dag (1 på morgonen, 1 vid middagstiden och 2 till natten)

### FÖRPACKNINGAR

Glas à 20 30 40 100 och 250 tabletter

Sjukhusförpackningar à 10×100 tabletter och  
5×250 tabletter

**LEO • HALSINGBORG**

Oral terapi vid allergiska rinit

# RINOMAR®

Vid rinit och sinuter av allergisk gen

allergisk genes har RINOMAR pålitlig avsvallande och sekretionshämmande effekt. Preparatet kan med fördel användas för långtidsbehandling eftersom effekten ej avtar och biverkningsfrekvensen förblir låg även vid långtids dagligt bruk. Genom avsvallningen och därmed förbättrad dränering av bihålor och mellanöron minskas risken för komplikationer i form av sinusit, otitis media, otosklingit etc.



Tabletter för vuxna — Mixtur för barn

## DEKLARATION

10 ml mixtur resp. 1 tablet innehåller  
Cinnarizin 10 mg + fylläpparens lösning 0,5 mg

## DOSERING

Vuxna 1 tabl. 3 ggr dagligt  
Barn 5—14 år 1 tabl. 11 10 ml mixtur morg. och kväll  
Barn 1—4 5 ml mixtur morg. och kväll

## FÖRPACKNINGAR

Mixtur 100 och 250 ml  
Tabletter 25, 100 och 250 t

Se följande FASS 1969



# INFLUENZA CAUSES GREAT LOSSES TO SOCIETY

The epidemic of influenza in 1957-1958 was calculated to have reduced the total volume of work during a year in Sweden by 0.29%—a loss of 5 million work-days, corresponding to about 9 million pounds. (1)

FLUMIDIN prophylaxis gives 40-94% protective effect during an influenza epidemic with high frequency of morbidity (2)

## FLUMIDIN®

KABI

### Results of clinical investigations (2)

Type of virus infection	Medication	Dosage schedule	Reported results
Influenza			
High morbidity (0-8%)	Prophylaxis	50 mg t.i.d.	55% protection
	Prophylaxis	200 mg t.i.d.	From 40% to 94% protection
Low morbidity (2-4%)	Prophylaxis	200 mg t.i.d.	From no observable effect to 78% protection
Upper respiratory infection	Prophylaxis	50 mg t.i.d.	From no observable effect to 5% protection
	Prophylaxis	200 mg t.i.d.	From 42% to 67% protection
	Prophylaxis	400 mg b.i.d.	From statistically not significant to 70% protection
Morbidity (measles)	Prophylaxis	500 mg t.i.d.	No observable effect
Vaccinia (chicken pox)	Prophylaxis	500 mg t.i.d.	No observable effect
Scarlet fever	Therapy (suppression)	200-500 mg t.i.d.	Statistically significant reduction of average illness period by about one third; from > 6 days to about 4 days
Upper respiratory infection	Therapy (suppression)	200 mg t.i.d. 400 mg b.i.d. t.i.d.	From no observable effect to statistically significant reduction of number of cases with illness period above 5 days
Herpes zoster	Therapy (suppression)	200 mg t.i.d. 400 mg b.i.d.	Rapid relief of pain and mitigation of neurological manifestations, inhibition of postherpetic
Morbidity (measles)	Therapy (suppression)	500 mg t.i.d.	No observable effect
Parotitis mumps	Therapy (suppression)	500 mg t.i.d.	No observable effect
Polio	Therapy (suppression)	50 mg b.i.d.	No observable effect
Vaccinia (chicken pox)	Therapy (suppression)	500 mg t.i.d.	No observable effect

Flumidin® 4 x 10 mg tablets & N,N-[Amino]drobus (2-hydroxyethyl)] biguanide hydrochlor (ABOB)

Indication Influenza (prophylaxis)

Dosage Adult and children over 5 years of age 400 mg twice daily (morning and evening). Children under 5 years of age 200 mg three times daily

Packs 100 and 500 tablets.

(1) K. H. B. 1957-1958 års influensapandemi i Sverige. S. Läkartidn. 27 (1959), p. 19.  
(2) Flumidin — samlade erfarenheter med ABOB. AB Kabi, Stockholm 1965 p. 8. (50 pages, partial English, available upon request from AB Kabi, Stockholm 30).

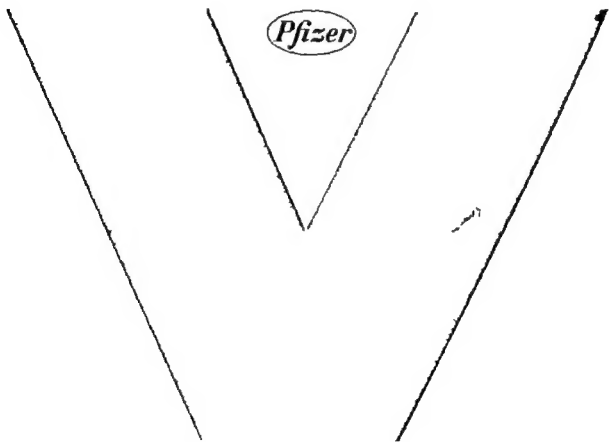
Further information is available on request from AB KABI Stockholm 30 S. den

AB KABI STOCKHOLM 30 SWEDEN

ett nytt antibiotikum

**Vicramycin<sup>®</sup>**  
doxycylin

endast en dos per dag



Nytt antibiotikum med unika egenskaper

